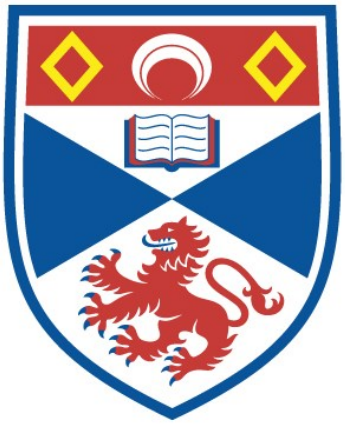
ETHANOL METABOLISM AND FLOODING TOLERANCE IN CONIFEROUS TREES

Margaret Anne Baines

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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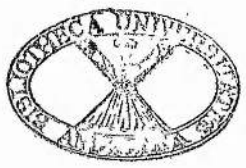

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M.Baines

ABSTRACT

Various indicators of flooding damage were investigated to assess their possible use as monitors of the effects of flooding. The effects of anoxia on root leakage and needle chlorophyll content were observed using seedlings of Pinus contorta and Picea sitchensis in water culture. Wood-cores were collected from the trunk bases of Pinus sylvestris trees on sites of various wetness at Tentsmuir, Fife, and their ethanol contents determined. Samples from trees growing in the wetter areas contained more ethanol than those from the dry areas. This information suggested that ethanol content was the most likely indicator of those investigated, to be worth examining for use as a monitor of anaerobic conditions and flooding damage.

Ethanol levels in wood cores could be related to those in the root, and so a useful field sampling method was established. Further cores were taken from trees of Pinus sylvestris and Picea abies on three sites at Tentsmuir, throughout the year. Ethanol could always be detected, but seasonal fluctuations could be observed. Concentrations were highest at times of high water table (i.e. low oxygen availability), and high metabolic activity (i.e. high oxygen demand).

In glasshouse experiments, seedlings of Pinus contorta (flood-tolerant) and Picea sitchensis (flood-intolerant), were maintained in aerated or unaerated water culture, and flooded or unflooded pots. Cessation of aeration caused an immediate increase in root ethanol concentrations in both species, but this was nine fold greater in Picea sitchensis, indicating a difference in response to anoxia by flood-tolerant and flood-intolerant species.

The toxic nature of ethanol was investigated in the same conditions, but with ethanol added to the culture medium. Root leakage, needle chlorophyll, root tip viability, and root respiration were investigated. Respiration data indicated that, unlike Picea sitchensis, Pinus contorta could metabolise ethanol in anaerobic conditions.

/cont.

It is suggested that flooding tolerance is due to a combination of the abilities to transport oxygen from the shoot to the root, to oxidise ethanol formed, and to control actual rate of glycolysis and ethanol production. Flood intolerant species do not possess these abilities, and may even suffer increased rate of ethanol production in anaerobic conditions.

The use of ethanol as a monitor of the effects of flooding and the observation of change in rate of ethanol production in anaerobic conditions as a method of screening seedlings for flooding tolerance, are exciting possibilities for the future.

Curriculum vitae

Date and place of birth: 17th August 1953, Dartford, Kent.

Education : 1971 - 1974 Bedford College, University of London.
Graduated in 1974 with First Class Honours in Botany.

Research : 1974 - 1977 Research Assistant in Botany Department,
University of St. Andrews.

CERTIFICATE

I hereby certify that Margaret Baines has been engaged upon research from October 1974 onwards under my supervision, to prepare the accompanying thesis for the degree of Doctor of Philosophy.

Professor R.M.M. Crawford

St. Andrews,

April 1978

DECLARATION

I hereby declare that the following thesis is based upon work carried out by me, that the thesis is my own composition and that it has not been previously presented for a higher degree.

The thesis is submitted to the University of St. Andrews under Ordinance General No. 12, and Resolution of the University Court 1964, No. 1, for the degree of Doctor of Philosophy.

The research work was carried out in the Department of Botany, University of St. Andrews, under the supervision of Professor R.M.M. Crawford.

St. Andrews,

April 1978

ACKNOWLEDGEMENTS

I would like to express my thanks to Professor R.M.M. Crawford for all his advice and encouragement over the last three years, his guidance has been invaluable. I also wish to thank Professor J.A. Macdonald, in whose department the work was carried out, and all the technical staff whose practical assistance has been much appreciated.

The Forestry Commission very kindly supplied tree seeds and seedlings, and allowed sampling to be carried out in their Tentsmuir plantation. The Nature Conservancy Council were also very co-operative, and allowed sampling at Morton Lochs Reserve, Tentsmuir.

The work was carried out during the tenure of an N.E.R.C. Research Studentship.

CONTENTS

INTRODUCTION

The economic importance of the effect of waterlogging on British timber forests is considerable. In Britain, some 50% of Forestry Commission land is subject to periodic or permanent waterlogging, and in current planting areas, the proportion may approach 100% (Toleman and Pyatt 1974). Trees may die under the influence of waterlogged soil, or may be lost through premature wind blow, since trees established on these wet sites often have shallow rooting systems (Fraser and Gardiner 1967; Day 1950). This characteristic shallow rooting has often lead to confusion. For example, many species of Spruce are found growing on muskeg soils, but this is not because they are tolerant of the anoxic conditions produced by the high water table, but because they avoid them by having shallow spreading rooting systems which do not penetrate the deeper waterlogged soil. Although it is true that these trees may become established, this is not a satisfactory situation because of the high risk of premature wind blow.

However, this point underlines the necessity to differentiate between true tolerance of anoxic root conditions as produced by flooding, and avoidance of root anoxia. Many plants avoid anaerobic root conditions by various morphological and anatomical adaptions. These are especially important in those species which grow in permanently waterlogged sites and need to be able to grow and reproduce under these conditions. The diffusion of oxygen from the overground parts of the plant to the roots is a well documented method of avoiding root anoxia, and has been demonstrated in many species, (Greenwood 1967). However, oxygen diffusion may not be as important as has often been assumed, since it is not clear to what extent this gaseous diffusion satisfies the total oxygen requirement of the roots (Greenwood 1967). An attempt to calculate this has been made by Vartapetian and Nuritidinov (1976), following careful observations, and they conclude that, compared with the total demand of the root for oxygen, the shoot to root diffusion

pathway is only capable of accounting for 8% of the total oxygen demands of the root. Since the apical 3-5 mm of the root tip exists in a state of permanent anoxia, even in an atmosphere of 21% oxygen (Crawford 1978), any reduction in the oxygen in its environment will aggravate the oxygen diffusion deficit of the apical tissues, which will then progress to the remainder of the root system.

It seems probable that the adaptive significance of the outward diffusion of oxygen from the roots is more likely to be in connection with the oxidation of reduced substances of a toxic nature, found in the rhizosphere, but this activity cannot be compared quantitatively with the oxygen demands of the root itself.

Whatever the precise role of shoot to root diffusion in overcoming the hazard of waterlogged soil, it appears to be of main importance in those species living in permanently waterlogged wetland habitats.

In considering the ability of forest trees to withstand waterlogging, the type of conditions under consideration, are generally those of periodic inundation In these circumstances it is the ability of trees with already well established, deep rooting systems to survive the period of inundation and resume normal growth afterwards which is important; rather than their ability to make positive growth during the period of waterlogging. Crawford (1972) has shown that flood tolerant and flood intolerant trees cannot be distinguished by their growth rate during flooding, but only by the ability of the flood tolerant species to resume an almost normal growth rate after flooding.

Since trees may have already well established, deep rooting systems at the time of inundation, it is unlikely that they will be able to quickly adapt anatomically to avoid anoxia, although new adventitious roots may be produced in some cases. Metabolic changes, however, may be induced very quickly, and it seems more likely that a metabolic process whereby flooding can be tolerated, comes into action.

It is known that certain trees are more tolerant of flooding than others, but this has usually been determined through many years of observation, and through the wasteful method of determining the force required to pull over the full grown tree. Clearly there is a need for a more efficient, less destructive method of monitoring the effects of flooding, and determining the inherent flood tolerance of tree species or varieties.

This thesis sets out initially to investigate the various indications, and symptoms of flooding damage found in trees, and to assess their possible use as monitors of the effects of flooding. Having done this, it was evident that ethanol content was the most likely candidate for use as a simple but accurate guide to the condition of trees in waterlogged soils, and that most unaffected by other external conditions. The production of ethanol was then studied more closely in the field throughout the year. Field observations were followed up by glasshouse experiments using tree seedlings. The glasshouse experiments undertaken also investigated the relationship between flood tolerance and ethanol production. Further work considered the possible nature of the toxic effect of ethanol produced *in vivo*, by observing the effects of ethanol added to the external medium.

The selection of a flood tolerant species or variety, simply by observing the ethanol production of the roots in drained and flooded conditions, would mark a major advance in forestry technique.

PLANT MATERIAL

Four conifer species were used throughout the work detailed in this thesis; *Pinus contorta* var. *latifolia* S. Watson; *Pinus sylvestris* L.; *Picea sitchensis* Carr. and *Picea abies* Karst. All these species are frequently planted on Forestry Commission land in Britain.

The species of Pinus contorta and Picea sitchensis used in the glasshouse experiments were all 2-3 year old seedlings, either grown in the Botanic Garden in St. Andrews from seed supplied by the Forestry Commission in Edinburgh, or supplied directly by them as seedlings. All were maintained in pots containing a 1:1 mixture of peat and sand, kept moist with tap water, and with periodic additions of nutrient solution. The seedlings were in a 16 hour day regime.

The trees of Pinus sylvestris and Picea abies were growing in mature stands, baring those referred to in Chapter I, which were from a younger stand. All were in the Forestry Commission plantation at Tentsmuir, Fife.

Origin of seedlings used:-

Pinus contorta

Provenance	65/797	71/7114/3
Latitude	46°20'-48°20'N	55°15'N
Longitude	c.124°W	127°W
Elevation	150 m	300 m
Habitat type	Coastal/ Washington	Hazelton

Picea sitchensis

Provenance	66/7111/1/3	70/7111
Latitude	54°0'N	54°0'N
Longitude	132°10'W	132°10'W
Elevation	150 m	150 m
Habitat type	Masset/Queen Charlotte Bay, B.C.	Masset/Queen Charlotte Bay, B.C.

METHODS

1. GENERAL METHODS USED THROUGHOUT

A. Nutrient solution

A nutrient solution was used as a root medium during periods of adjustment to water culture before experiments were carried out with the roots in distilled water. Seedlings were maintained in nutrient culture (with various concentrations of ethanol added) throughout experimental series F (see section 4/D below), since this was for a duration of five weeks. Pot grown plants were also treated with the nutrient solution approximately every two weeks. In all cases a modified Hoagland's solution (Epstein 1972) was used, at one tenth strength, and adjusted to pH 6 using Molar Potassium hydroxide. There was no appreciable pH drift during the relatively short readjustment periods, and in experimental series F (see section 4 below), the solutions were renewed at weekly intervals.

B. Oxygen

Oxygen was determined using a revised Winkler method (Golterman 1969). Except for Experimental series B (section 2/B below), samples always had to be taken from narrow necked containers. It was important that there was as little disturbance as possible, and that the procedure was carried out quickly, especially when sampling sealed containers. For these reasons, samples could not be collected by the usual method of immersing and sealing the sample container in the water to be analysed. Instead, 25 ml of water were drawn off quickly with a pipette, and carefully transferred to a 25 ml screw top container, which was immediately sealed. The analysis then proceeded in the usual manner. Although some accuracy may have been lost, oxygen measurements obtained are likely to be higher, rather than lower than the true value. The low oxygen concentration found to prevail in the sealed bottles is therefore a maximum rather than minimum level.

C. Glasshouse conditions

All glasshouse experiments were carried out under glass at approximately 20°C in 16 hour days, using 2-3 year old pot grown seedlings.

2. ROOT LEAKAGE UNDER ANOXIA

Methods used in all cases:-

i) Potassium - Flame Photometer

ii) Conductivity - Conductivity meter

iii) Amino acids - Hydrindantin method (Colwick and Kaplan 1957). This method is specific for amino acids, and interference is only caused by ammonia.

iv) Glucose - determined enzymatically using hexokinase and glucose-6-phosphate dehydrogenase (Bergmeyer 2nd edition 1965).

v) Total carbohydrates - the Anthrone method (Allen 1974)

vi) Root volume - In several cases, results are expressed in relation to root volume. This was measured by displacement of water.

A. Experimental series A

Short term water culture of Pinus contorta and Picea sitchensis in sealed and unsealed bottles.

The experiments involved 2-3 year old seedlings of Pinus contorta (provenance 65/797) and Picea sitchensis (provenance 66/7111/1/3). Sixteen seedlings of each species were initially set up in open aerated 2 litre jars filled with nutrient solution. They were kept here several days, to adjust to water culture conditions. After two changes of distilled water, the roots were surface sterilised in 0.02% Mercuric chloride, and placed in sterilised distilled water in sterilised, blackened 600 ml bottles. Eight bottles for each species were sealed with bungs around the seedling stems, the seal completed with vaseline. The other eight bottles were aerated using air stones, and the seedlings supported

by cotton wool. Two bottles of each species in each treatment (i.e. a total of eight bottles) were "harvested" after 3,6, 12 and 24 hours. Initially, samples were taken to determine dissolved oxygen, and subsequently further samples of the water culture solution were removed to determine pH, coductivity, and potassium, amino acids, glucose, and total carbohydrate concentrations.

2. B. Experimental Series B

Water culture of Pinus contorta and Picea sitchensis in aerated or non-aerated open jars.

The tree seedlings used were from the same provenances as those used in 2/A above. Three seedlings of each species were transferred after washing from nutrient culture to distilled water culture in open 2 litre jars. The jars were aerated using air stones for 6 days, the pumps were then turned off.

Conductivity of the water culture solution was measured 24 and 48 hours after the experiment was set up, and 24 and 48 hours after the cessation of aeration.

2. C. Experimental Series C

Two week water culture of Pinus contorta and Picea sitchensis in sealed bottles also containing various concentrations of ethanol.

The trees used were 2-3 year old seedlings of Pinus contorta (provenance 65/797) and Picea sitchensis (provenance 70/7111). Twelve seedlings of each species were set up in sterilised blackened 600 ml bottles as in 2/A above. All bottles were sealed except for two for each species, where the seedlings were in aerated distilled water.

The contents of the other bottles were as follows:-

2 bottles for each species:
- distilled water
- 10^{-1}M ethanol
- 10^{-2}M ethanol
- 10^{-3}M ethanol
- 10^{-4}M ethanol

Samples of the culture solutions were removed before the beginning of the experiment, and then after 1,2,3,5,7 and 14 days, being topped up with the appropriate ethanol solution at these times. The samples were used to determine potassium content, conductivity and pH. Root volumes were determined, and results expressed in relation to these, giving a more accurate measure of root leakage per unit root area.

3. CHLOROPHYLL - EXTRACTION AND ANALYSIS

Leaves were sampled for chlorophyll determination after 3 and 6 days aeration, and 1 and 8 days after the cessation of aeration in experimental series B (see 2/B above). Leaves were also sampled in experimental series C (see 2/C above), after 2,4 and 14 days of aeration and non-aeration. Fresh weights and needle areas were determined. Chlorophyll content is probably more accurately expressed as mg/mm^2 than mg/g (fr.wt.) since, during flooding, there is a decrease in water absorption (Kramer 1951b). This leads to a reduction in water content, and hence fresh weight of the leaves. The area of the leaves, however, remains relatively unchanged.

The method for chlorophyll extraction and spectrophotometric analysis was that employed by T.W. Goodwin (1965). Calculations were based on those of Maclachlan and Zalik (1965).

4. ETHANOL AND MALATE

A. Extraction and Analysis

i) Root extraction procedure -

a) Fibrous roots from seedlings: The roots were harvested without washing (as this would remove ethanol), blotted dry, and extracted by grinding with 6% perchloric acid and sand. The extracts were then deproteinised (Crawford 1977).

b) Woody roots from forest trees: Root samples collected in the field were sealed in polythene bags, and on return to the laboratory, were pared into shavings for extraction in the same manner as the wood cores.

ii) Wood core collection and extraction procedure -

Cores of wood 0.5 cm in diameter traversing the base of the trunk were removed using a tree borer. Cores were removed in duplicate from each of five trees at every site (see Chapter II). The 5 trees at site 129 (fig. II/1) were marked throughout the sampling. Trees from the homogenous stands in sites 36 and 47 (fig. II/1) were sampled randomly until May when marking was carried out. On returning to the laboratory, the wood samples were fragmented and soaked in 6% perchloric acid for 2 hours at 0°C. The wood fragments were then gently agitated in the cold for a further 30 minutes with fresh perchloric acid. This last extraction always contained less than 5% of the wood ethanol content. The perchloric acid was removed from the extracts (Crawford 1977) and they were then assayed for ethanol.

4. B. Experimental series D

Short term water culture of Pinus contorta and Picea sitchensis in sealed and unsealed bottles, to determine ethanol concentration in the roots.

Twenty-four seedlings each of Pinus contorta (provenance 65/797) and Picea sitchensis (provenance 66/7111/1/3), were transferred from pots (after washing the roots) to sterilised blackened bottles containing sterile water (as in experiment A). Half of the bottles for each species were sealed, and the others aerated. Oxygen content was monitored.

As water culture, even when aerated, gave signs of increasing the extent of anoxia in roots of Picea sitchensis, a second series of flooding experiments was carried out in which the plants were flooded while growing in their pots in a 1:1 mixture of peat and sand.

Three trees of each species in each treatment were sampled after 3, 6, 12, and 24 hours and analysed for ethanol content. The investigation required the estimation of the ethanol concentration in the roots. The ethanol in the culture medium was not included in the results.

A subsidiary experiment using more Picea sitchensis seedlings in open but unaerated water culture in 2 litre jars, was conducted, and roots sampled and analysed for ethanol after 72 hours.

4. C. Experimental series E

Root tip viability in Pinus contorta (provenance 65/797) and Picea sitchensis (provenance 66/7111/1/3) after root immersion in ethanol.

Eight trees of each species were set up in open 2 litre jars containing various concentrations of ethanol:

2 seedlings of each species in each
- water
- 2×10^{-2}M ethanol
- 2×10^{-1}M ethanol
- 1M ethanol

Root tips were sampled after 1 and 7 days. The tips were immersed in dishes of 2-3-5 triphenyl tetrazolium chloride. This dye is turned from colourless to red by any oxidising activity, and it can therefore be used as a measure of the viability of the root tips.

4. D. Experimental series F

Five week water culture of Pinus contorta and Picea sitchensis (provenances 71/7114/3 and 70/7111 respectively), to determine the effect of exogenous ethanol on root respiration.

Two sets of the apparatus pictured in the figure were used, one as shown, and one in which oxygen free nitrogen was bubbled through. The bottles with trees, connected to each aspirator, contained the same ethanol concentration as that in the aspirator, but made up in nutrient solution.

A preliminary experiment was run without trees, monitoring the ethanol contents in the bottles, and from this it was determined to renew the solutions in both sample bottles and aspirators, weekly.

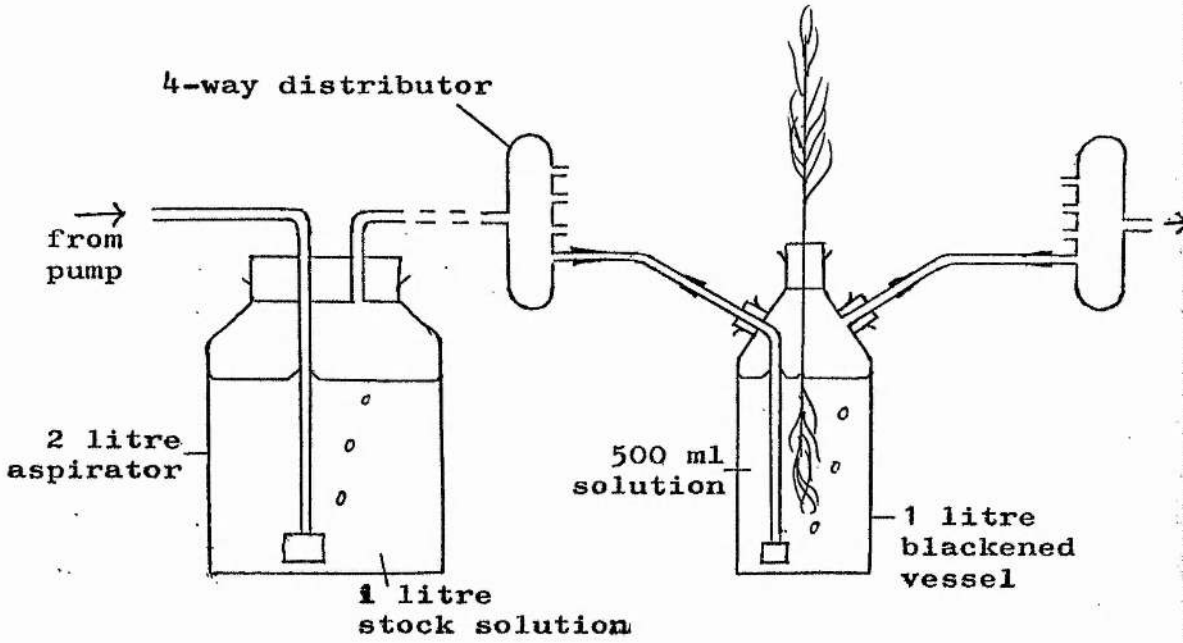
The trees were harvested after 5 weeks. Measurements of root segment respiration were made using the Warburg constant volume manometer. Approximately 2g fresh weight of root were used for each determination. Dry weights were found after the experiment. After sufficient readings were obtained to calculate $QO2$[*1] and QCO_2[*2], the flasks not containing KOH were flushed with nitrogen gas for 10 minutes. Readings were then taken to obtain values for $QCO_2(N_2)$[*3].

*1 (oxygen uptake in µl/h/mg dry wt.)

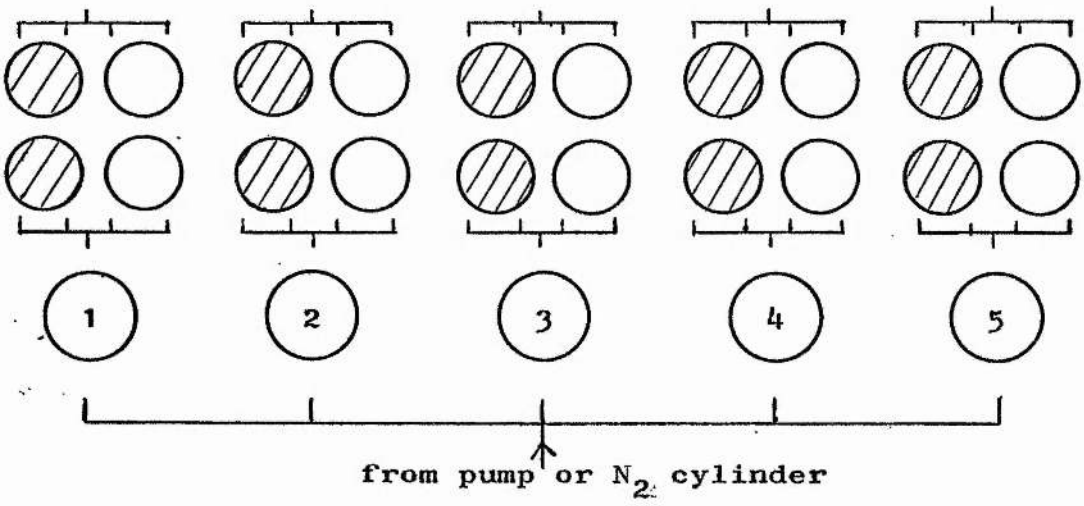
*2 (carbon dioxide evolution in µl/h/mg dry wt.)

*3 (Anaerobic evolution of carbon dioxide in µl/h/mg dry wt.)

Apparatus for Experimental Series F



Schematic diagram of experiment :-



Blackened vessels with Pinus contorta seedlings

Blackened vessels with Picea sitchensis seedlings

Aspirators

1 - water
2 - 10^{-4}M ethanol
3 - 10^{-3}M ethanol
4 - 10^{-2}M ethanol
5 - 10^{-1}M ethanol

CHAPTER I

SYMPTOMS AND INDICATORS OF FLOODING DAMAGE

1. INTRODUCTION

Much research has been carried out to document the deleterious effects that flooding has on various plant species (Childers and White 1942; Hunt 1951; Jackson and Campbell 1976; Kramer 1951b; Orlov 1962; Parker 1950; Watson, Lapins and Barrow 1976). However, some measurement is required which will give an easily acquired, but accurate representation of the state of health of a plant, or more specifically in this case, a tree, in a given set of soil water conditions. In this chapter various parameters which are known to be affected by flooding are discussed, and some are further investigated - leakage from the roots, leaf chlorophyll content, and ethanol production. These investigations were carried out both to ascertain which, if any, of the symptoms looked at, would give a good and readily attainable measurement of the condition of the tree, and also for the general information these investigations would give on the nature of flooding damage.

The symptoms of flooding damage described by previous workers include -

a) Reduction in growth rate (Cannon 1920; Childers and White 1942; Hunt 1951; Lees 1972; Leyton and Rousseau 1958; Vester 1972; Yu, Stolzy and Letey 1969).

b) Reduction in root: shoot ratio (Lees 1972; Vester 1972)

c) Wilting, reduction in water absorption and transpiration (Childers and White 1942; Hagan 1950; Kramer 1951b; Leyton and Rousseau 1958; Livingstone and Free 1917; Parker 1950; Vester 1972).

d) Chlorosis, leaf fall, and leaf epinasty (Crawford 1966; Hoveland and Webster 1965; Khotianovich 1958; Kramer 1951b; Kramer and Jackson 1954; Yamasaki 1952; Yu, Stolzy and Letey 1969).

e) Reduction in nutrient uptake, and leakage of substances from the roots (Christiansen et al 1970; Hiatt and Lowe 1967; Leyton and Rousseau 1958; Orlov 1962).

f) Increase in ethanol content of the roots (Crawford 1966; Crawford 1967; Crawford 1972; Bolton and Erickson 1970; Fulton and Erickson 1964; Grineva 1963; Lundegardh 1948).

While measurement of growth rate gives a very good indication of how trees are faring on different sites, this, and measurements of root: shoot ratio are not very practical methods for forestry workers to use for the quick determination of the viability of a tree in the current soil water conditions. The method at present used by forestry workers is rapid, if rather drastic; the practice is to find out how much force is required to pull the tree over (Fraser & Gardiner 1967). This gives a measure of the depth of penetration of the roots. However, this is a destructive analysis, not allowing for the continued monitoring of trees during and after improvements to the site such as new drainage patterns, have been made.

Wilting and epinasty are not readily observable phenomena in conifer species, and whilst diminishing transpiration can give a good picture of the effect of flooding (Parker 1950), this is again a long term proposition. It may be possible to measure the extent of chlorosis in controlled conditions, and this will be considered in section 3 of this chapter. However, the use of this measurement in the field immediately suggests various difficulties, for example, sample uniformity, and the rapid breakdown of chlorophyll in detached leaves.

Reduction in nutrient (and water) uptake is also difficult to measure in the field. Orlov (1962) made his observations by adding P^{32} to the soil, and monitoring its uptake. Vlamis and Davis (1944) looking at rice and barley, were able to take samples and analyse the expressed sap for potassium. It is possible that samples of sap could be taken from trees (Crawford 1972) and monitored for salt content. However, the levels found in the sap are likely to vary greatly between sites and the variations found would not be due, in the most part, to variations in the soil oxygen availability, but to other environmental factors.

Since the primary effect of flooding is the induction of an anaerobic root regime, a good indicator of flooding would be one which changed under these specific root conditions. Lack of soil oxygen results in an increase in the anaerobic respiration of the roots, and it has been shown (Bolton and Erickson 1970; Crawford 1966; Hageman and Flesher 1960) that under these conditions, easily measurable amounts of ethanol are formed. The measurement of ethanol may also help to elucidate the effects that the flooding is actually having on the roots themselves.

2. ROOT LEAKAGE UNDER ANOXIA

Although it has been frequently noted that root "exudation" can often be detected in "normal" conditions (Katznelson et al 1955; Rovira 1955), it has also been shown by other workers (Christiansen et al 1970; Hiatt and Lowe 1967; Marschner et al 1966; Vlamis and Davis 1944), that this exudation, or "leakage" is increased in anaerobic conditions. This phenomenon was therefore investigated for Pinus contorta and Picea sitchensis in water culture; initially in two short term experiments ("Experimental series A and B). A longer term experiment was carried out at a later date, the full results of which are discussed in Chapter IV. However, the results of this latter experiment which are relevant to the investigation here are also included in the following account.

Oxygen levels were monitored in EXPERIMENTAL SERIES A (fig. I/1). EXPERIMENTAL SERIES C was conducted under similar conditions. EXPERIMENTAL SERIES B was carried out using unaerated pots open to normal diffusion of the air. The level of oxygen measured during another experiment using this apparatus showed that in this case, oxygen levels only fell to about 50% oxygen saturation (fig. I/2).

Fig. I/1

Decrease in oxygen content of culture solution when aeration ceases and culture vessels are sealed.

● The mean value for four vessels containing Picea sitchensis

▲ The mean value for four vessels containing Pinus contorta

The vertical bars represent the maximum and minimum values recorded.

Dissolved oxygen in water around roots of Pinus contorta and Picea sitchensis seedlings in sealed bottles.

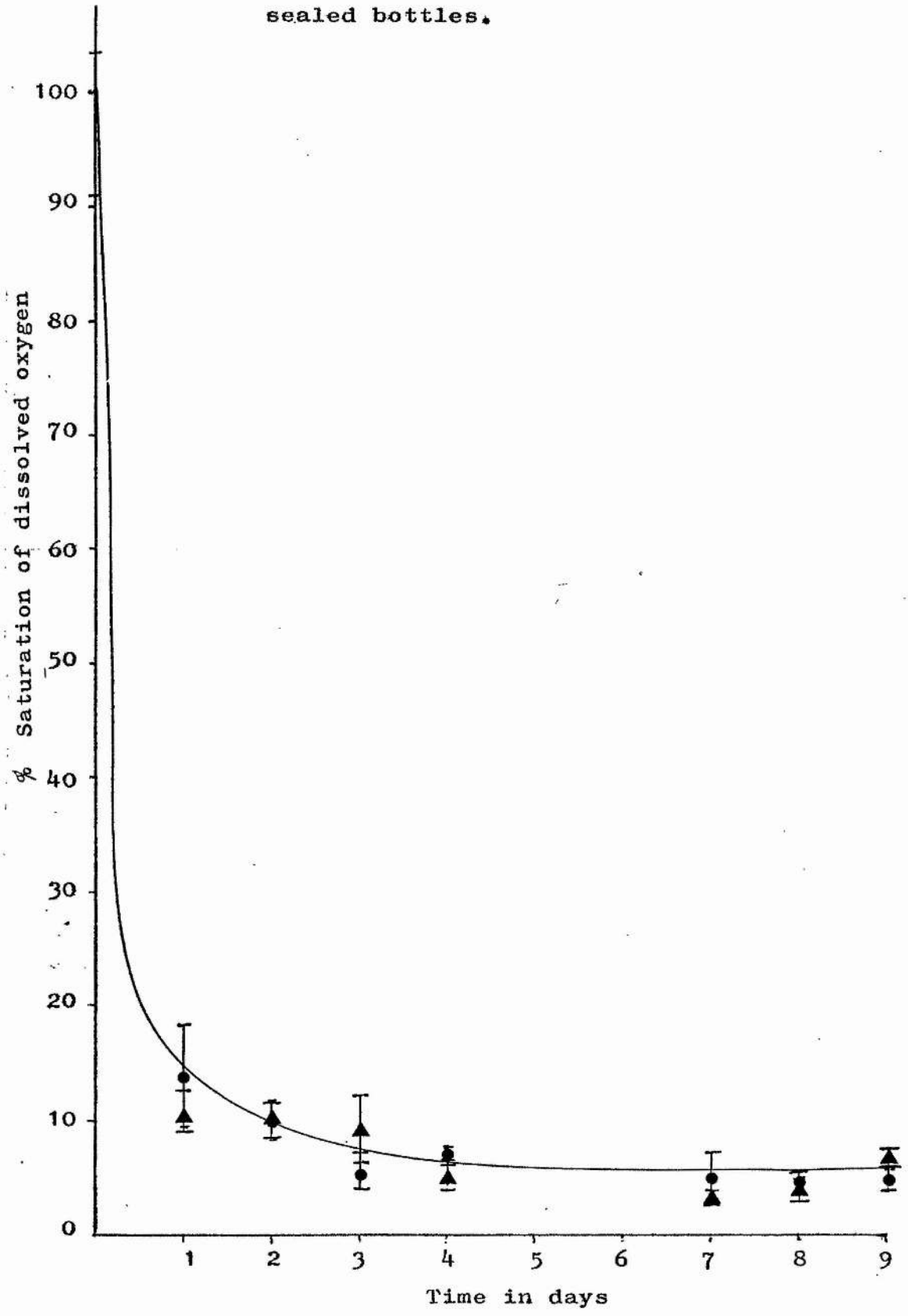


Fig.I/1

Fig. I/2

Decrease in oxygen of culture solution in open 2 litre jars when aeration ceases

● The mean value for two vessels containing Picea sitchensis

The vertical bars represent the maximum and minimum values recorded

Dissolved oxygem in water around roots of Picea sitchensis in open jars

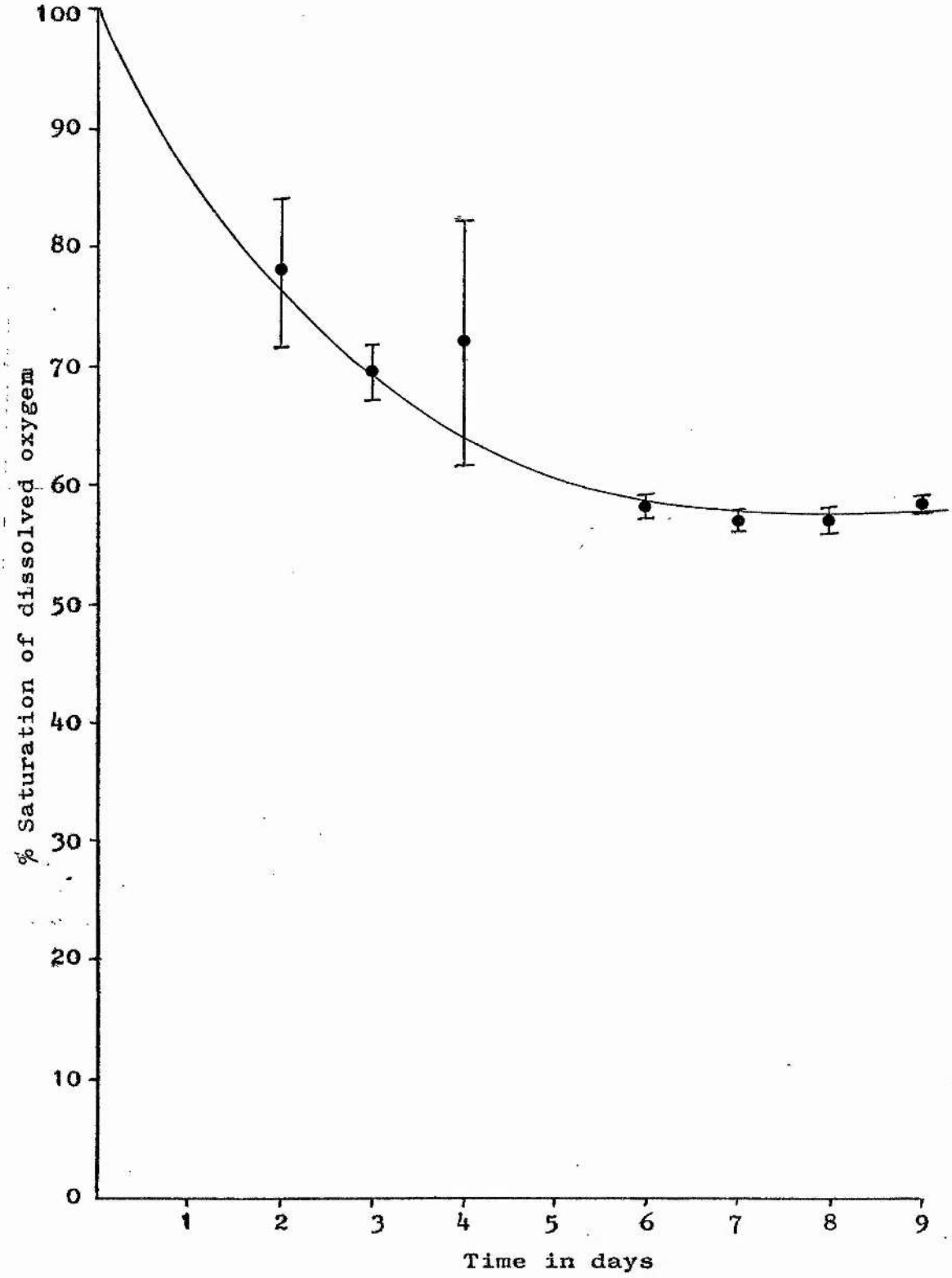


Fig. I/2

Fig. I/3

The potassium content (mg l^{-1}) of medium around roots of tree seedlings in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels.

▲ Pinus contorta Experimental Series C

△ Pinus contorta Experimental Series A

● Picea sitchensis Experimental Series C

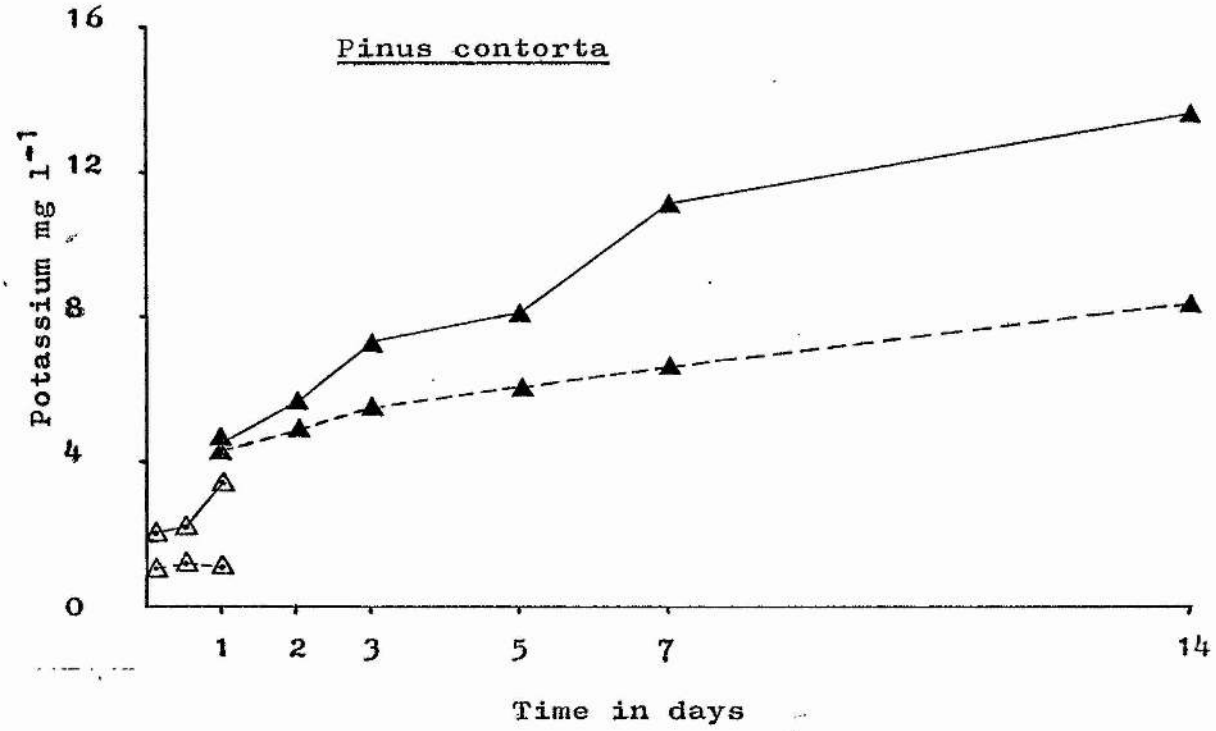
○ Picea sitchensis Experimental Series A

– – – aerobic
——— anaerobic
} in each case

Each point represents the mean for 2 culture vessels.

Potassium content of medium around

roots of tree seedlings in water culture



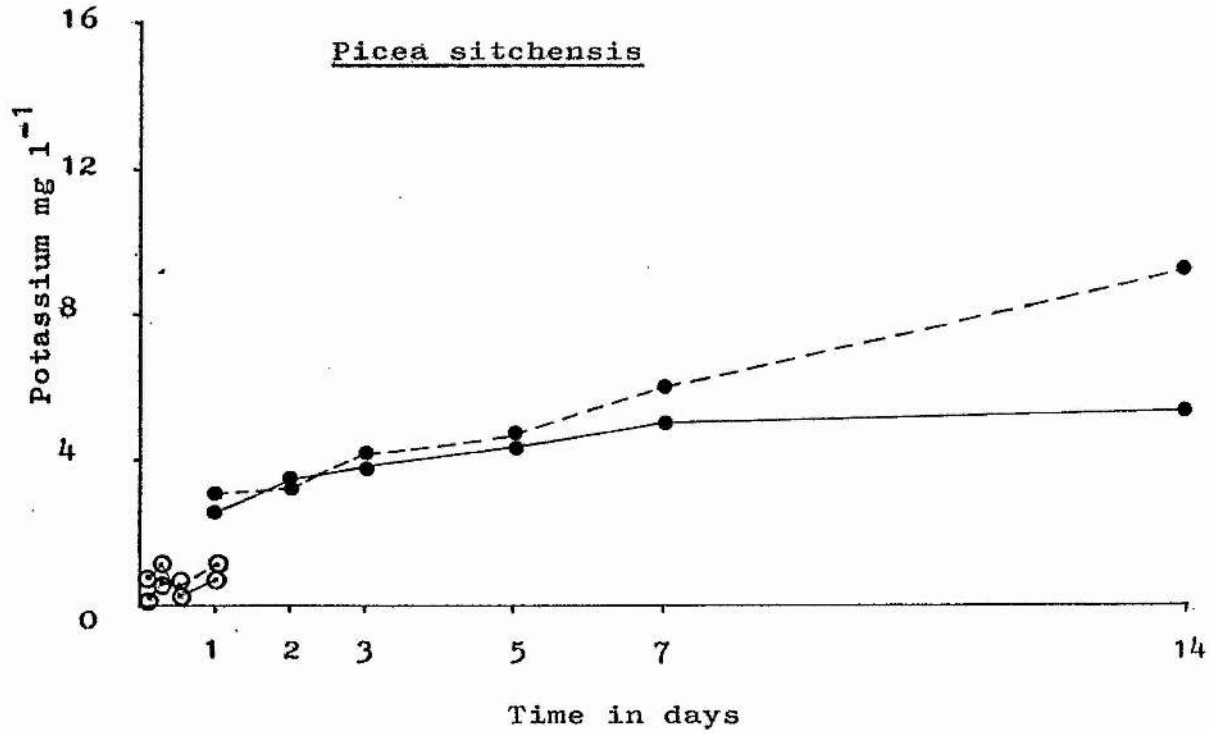


Fig.I/3

Fig. I/4

Histograms of potassium content (mg l^{-1}/cc root/day) of medium around roots of seedlings of Pinus contorta and Picea sitchensis in water culture in sealed (anaerobic) or unsealed aerated (aerobic) 600 ml vessels.

Experimental Series C.

Each column represents the mean value for two culture vessels.

Potassium content of medium around

roots of tree seedlings in water culture

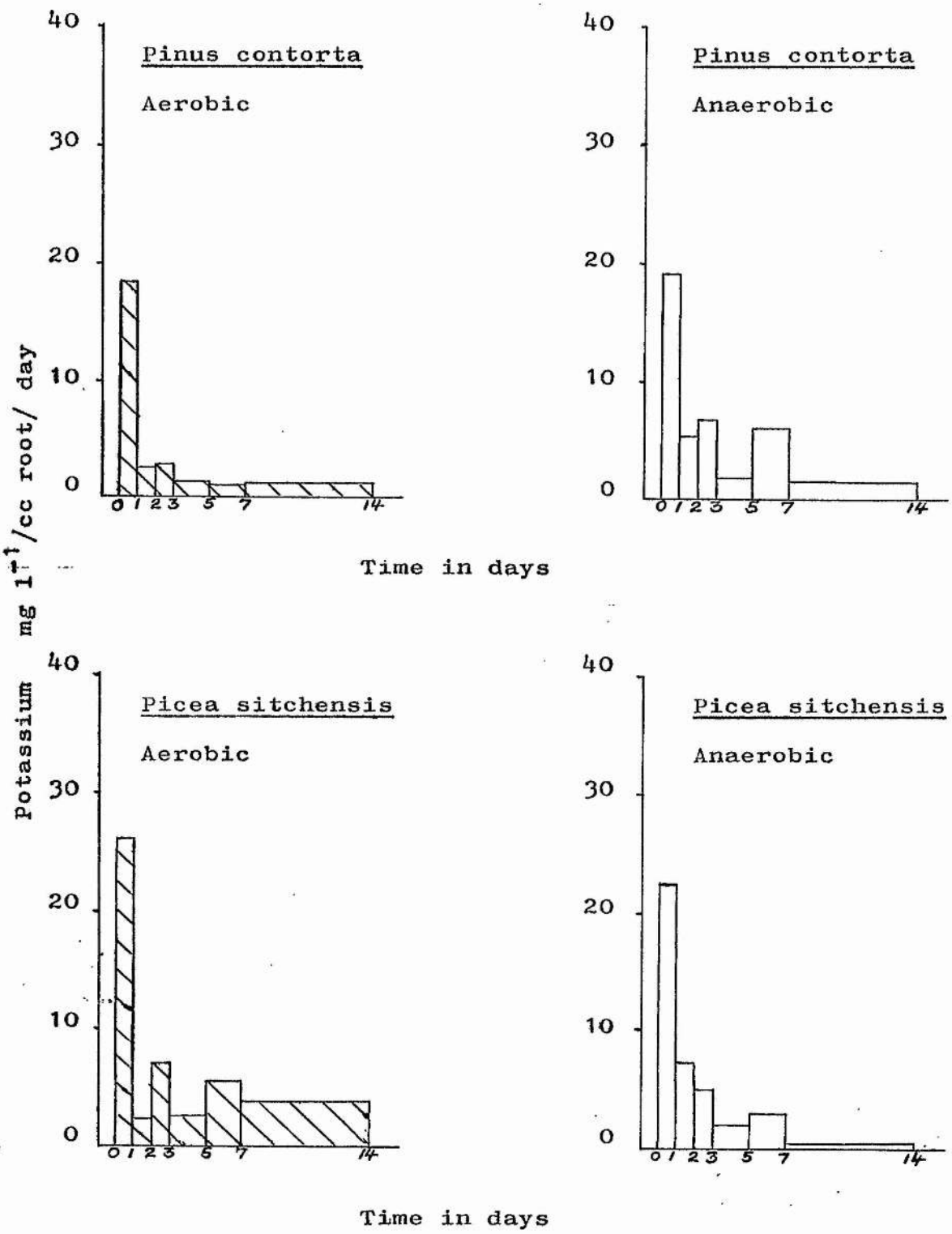


Fig.1/4

Fig. I/5

Conductivity (μmho) of medium around roots of tree seedlings in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels. (Experimental Series A and C); or in open and unaerated 2 litre jars (Experimental Series B).

▲ Pinus contorta Experimental Series C

△ Pinus contorta Experimental Series A

• (on same graph), Pinus contorta Experimental Series B.

ó Picea sitchensis Experimental Series C

O Picea sitchensis Experimental Series A

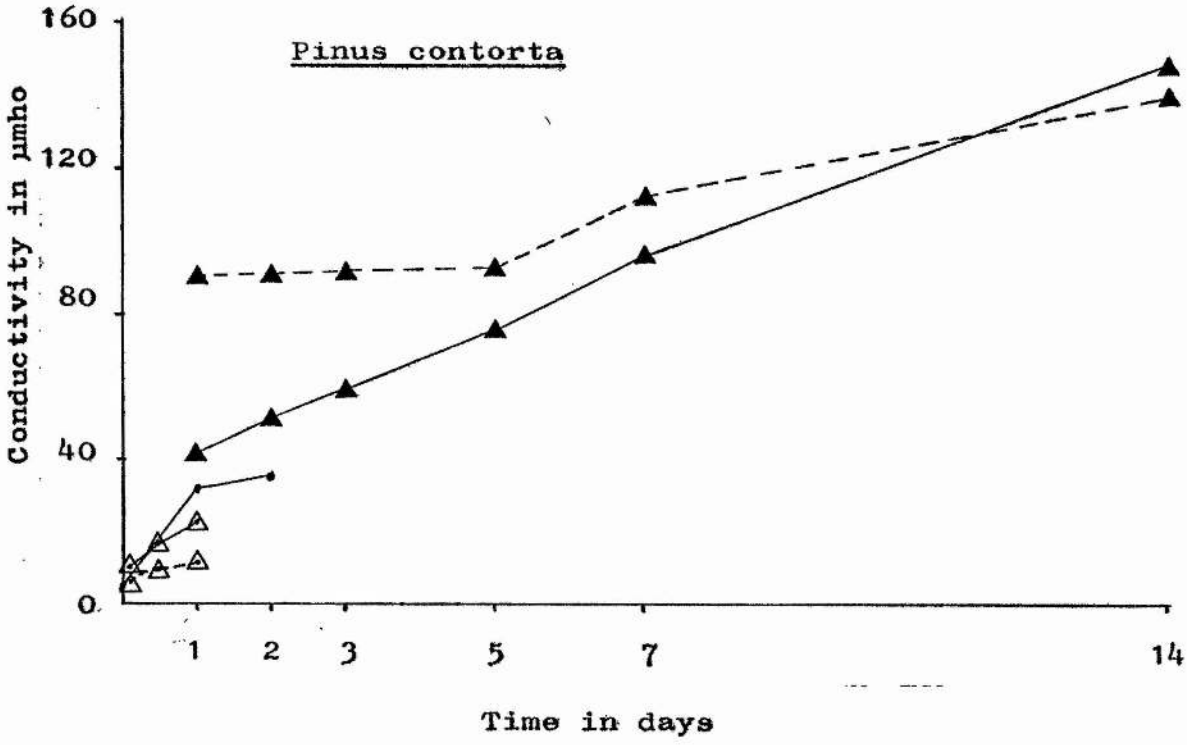
• (on same graph), Picea sitchensis Experimental Series B.

– – – aerobic
——— anaerobic } in all cases

Each point represents the mean value for 2 (Experimental Series A and C), or 3 (Experimental Series B) culture vessels.

Conductivity of medium around roots

of tree seedlings in water culture



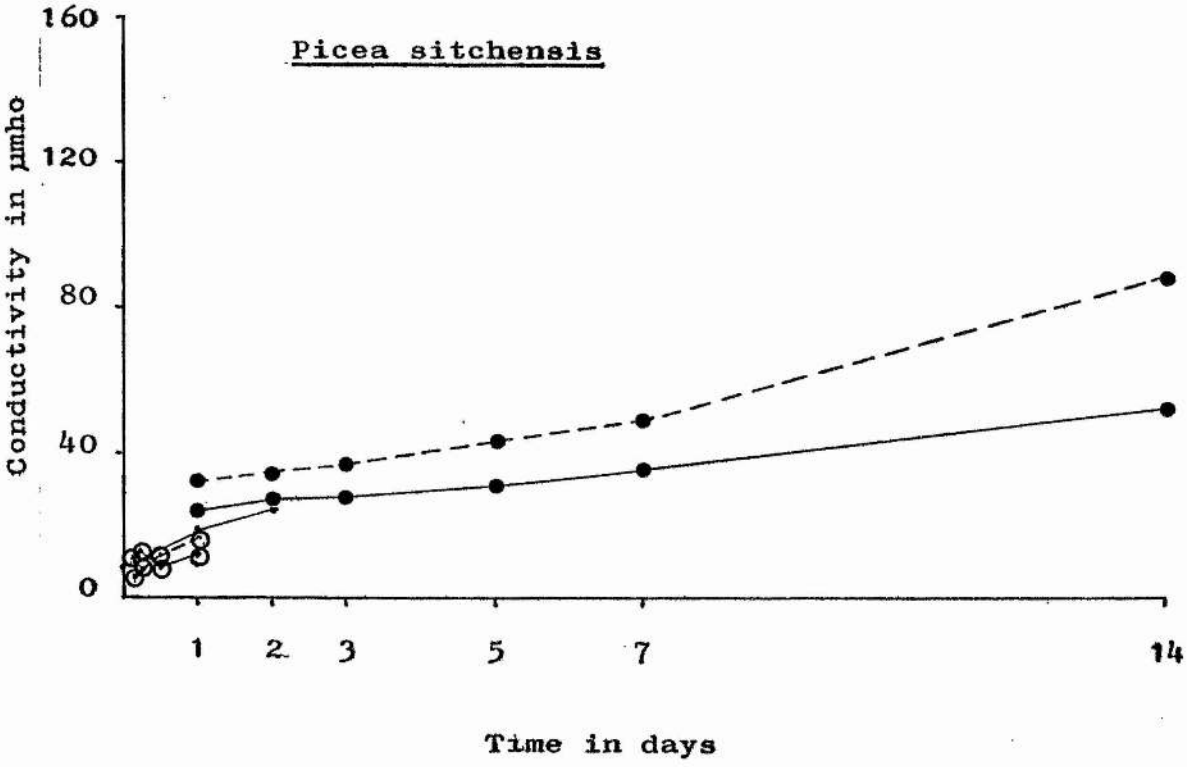


Fig.I/5

Fig. I/6

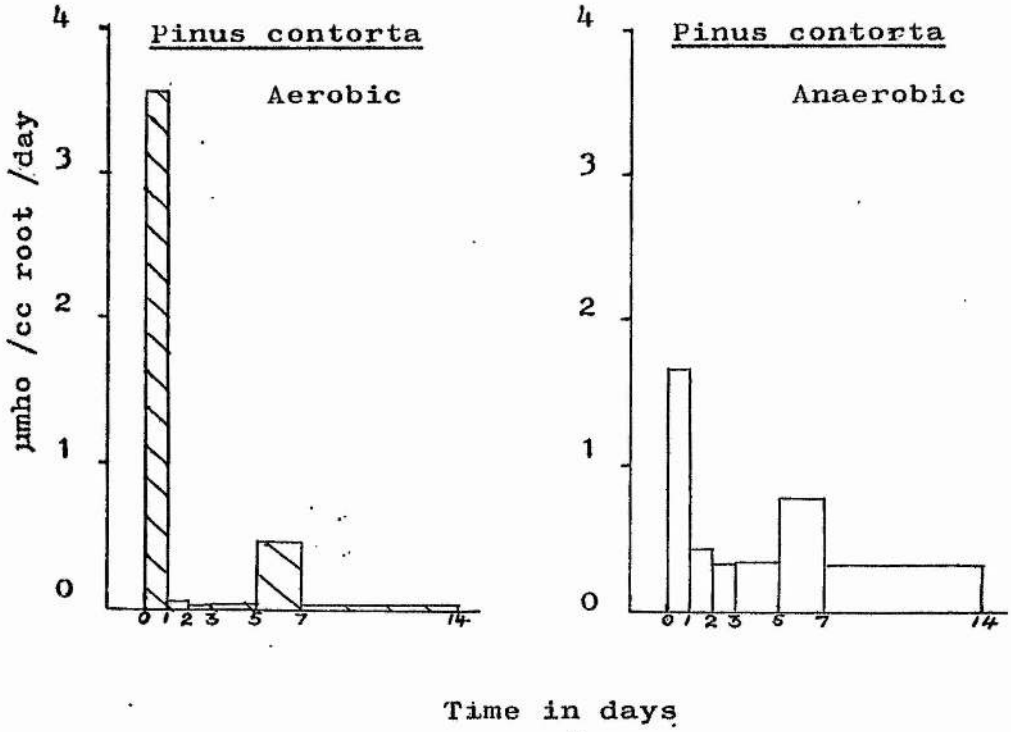
Histograms of conductivity (μmho/cc root/day) of medium around roots of seedlings of Pinus contorta and Picea sitchensis in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels.

Experimental Series C

Each column represents the mean value for two culture vessels.

Conductivity of medium around roots

of tree seedlings in water culture



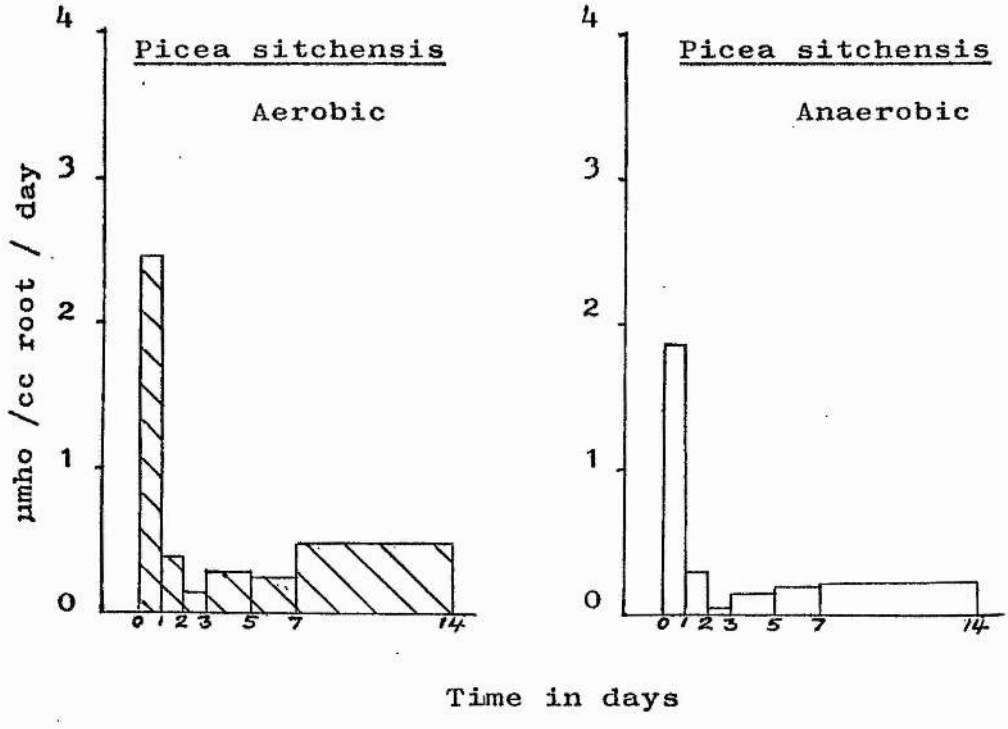


Fig.I/6

Fig. I/7

pH of medium around roots of tree seedlings in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels.

▲ Pinus contorta Experimental Series C

△ Pinus contorta Experimental Series A

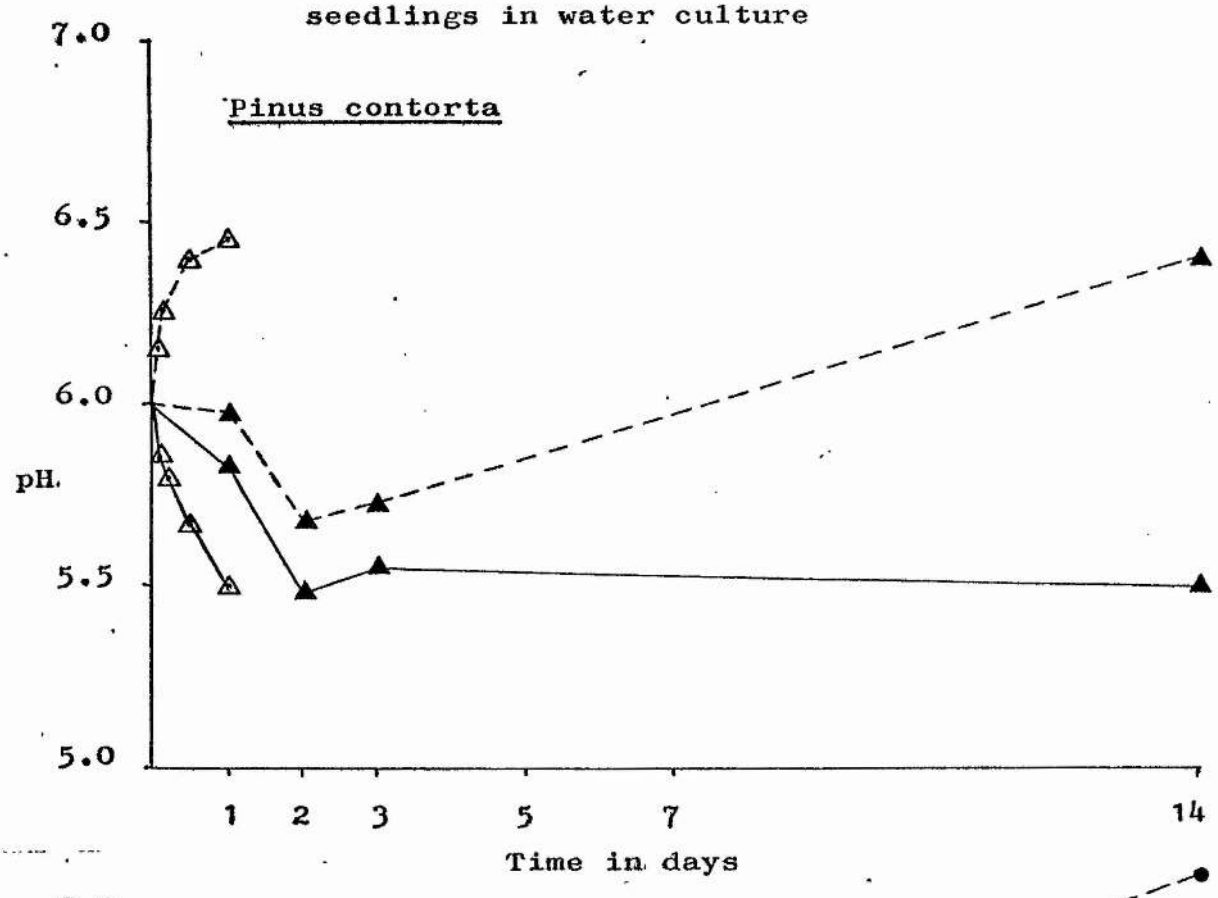
● Picea sitchensis Experimental Series C

○ Picea sitchensis Experimental Series A

- - - aerobic
——— anaerobic
} in each case

Each point represents the mean value for two culture vessels.

pH of medium around roots of tree seedlings in water culture



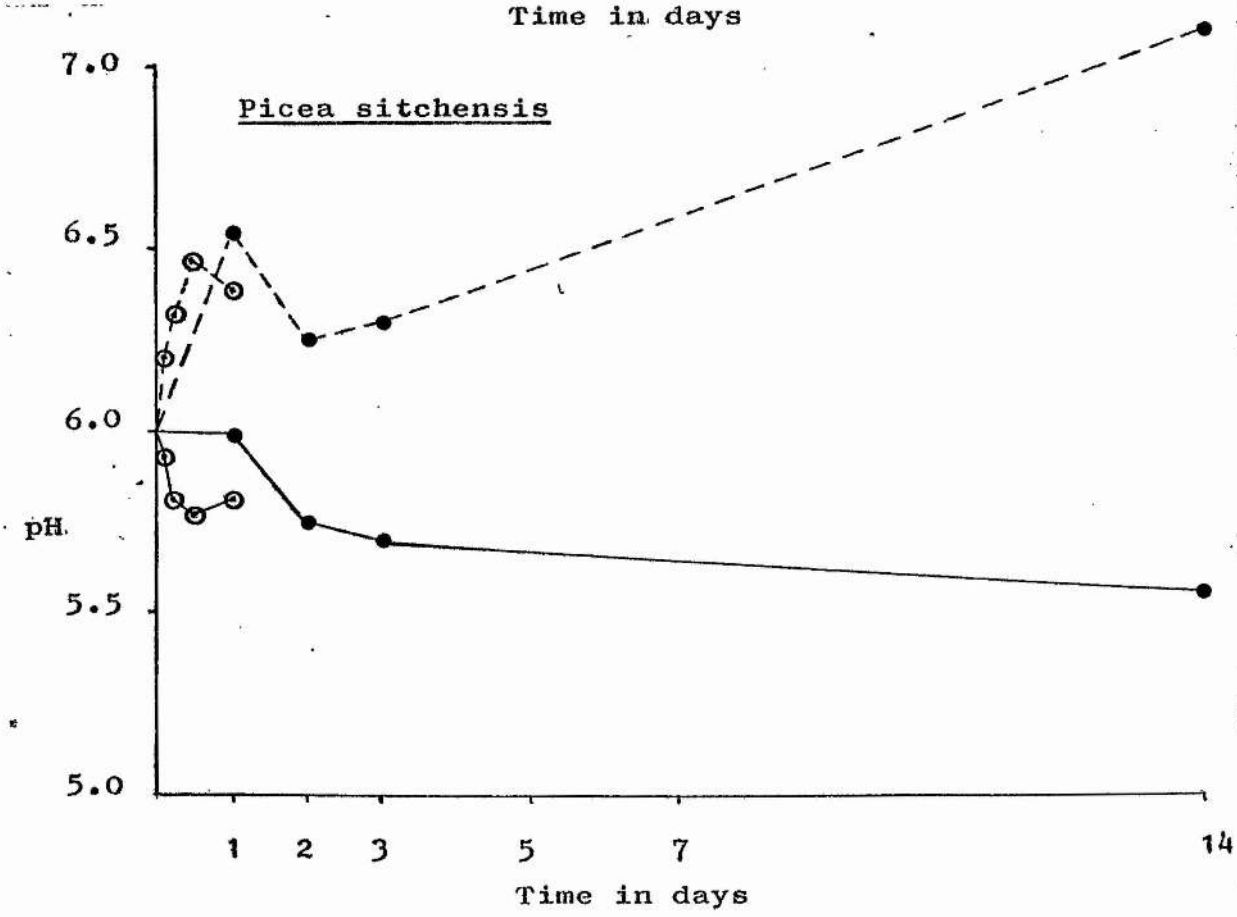


Fig.I/7

2. A. Results

The results of these experiments express "leakage" from the roots of Pinus contorta and Picea sitchensis in terms of -

a) Potassium concentration

b) Conductivity

c) pH

These measurements all refer to the external root medium.

POTASSIUM - EXPERIMENTAL SERIES A: In this initial short-term experiment, it could be seen that 24 hours after the cessation of aeration, the level of potassium in the medium around the roots of Pinus contorta seedlings, had risen to 3.35 mg/1, whereas the level from the aerated Pinus contorta root media does not rise above 1.26 mg/1. In Picea sitchensis however, there is no differentiation between the treatment, and the level of potassium in the media is, overall, lower than that found with Pinus contorta, not rising above 1.06 mg/1. (fig. I/3).

POTASSIUM EXPERIMENTAL SERIES C: Here there is again a clear differentiation between aerobic and anaerobic treatment in Pinus contorta. Potassium concentration reaches 13.6 mg/1 after 14 days anaerobiosis, but only 8.35 mg/1 after 14 days aerobiosis. The rate of potassium leakage (after the initial rise in the first day) is in fact about twice as high in the anaerobically treated plants (0.69 mg 1^{-1}/day) as compared to those treated aerobically (0.35 mg 1^{-1}/day). In this experiment, results are also expressed as mg/1/cc root in the form of histograms (fig. I/4), which show that, whilst in both treatments there is a high initial level of potassium leakage the subsequent continued leakage remains at a higher level in media around anaerobically treated plants of Pinus contorta.

As in Exp. Ser. A, there is no clearly defined response of potassium leakage from Picea sitchensis roots to anaerobic conditions. Leakage from the aerobically treated plants after 14 days is in fact higher (9.25 mg/1) than from the anaerobically treated plants (5.45 mg/1). The rate of potassium loss (after

the first day) is higher from aerobically treated plants (0.48 mg l^{-1}/day) as compared to that from anaerobically treated plants (0.25 mg l^{-1}/day). Both these values are low compared to those for Pinus contorta, if expressed in this form; however, when the results are expressed as mg/l/cc root, then the level of potassium loss appears to be of the same order of magnitude in both species. For Picea sitchensis as opposed to Pinus contorta, the histograms show a continued higher level of exudation in aerobically rather than anaerobically treated plants.

CONDUCTIVITY - EXPERIMENTAL SERIES A: Conductivity of the medium is related to time and treatment in Pinus contorta, reaching 21.76 µmho after 24 hours of anaerobic treatment, but only 11.76 µmho after 24 hours of aerobic treatment. Once more, the effect of anaerobic treatment on Picea sitchensis is not clear - conductivity rising in both treatments, and after 24 hours, reaching 12.92 µmho in anaerobic conditions, and higher, 17.82 µmho in aerobic conditions. (fig. I/5).

CONDUCTIVITY - EXPERIMENTAL SERIES B: This experiment was primarily conducted to observe chlorophyll levels (section 3, this chapter). However, some conductivity measurements were also made, and these are seen to agree well with those found in EXP. SER. A and EXP. SER. C. (fig. I/5). The trees used are of the same provenances used in EXP. SERIES A, but, as previously mentioned, the experiment was conducted in water culture in open jars rather than sealed bottles.

CONDUCTIVITY - EXPERIMENTAL SERIES C: The differences between aerobic and anaerobic treatments are not so marked in Pinus contorta in this longer term experiment. After 14 days, conductivity reaches 148.5 µmho in anaerobic treatment and 140 µmho in aerobic treatment. For the first 7 days of the experiment, conductivity is in fact higher in the media around the roots of aerobically treated plants. However, the histograms expressed in µmho/cc root (fig. I/6) show that this is due to a higher initial increase in conductivity (after 1 day) in the aerobic treatment; there is little further increase after this. This is confirmed

by the fact that the rate of increase in conductivity (after the initial rise during the 1st day) is more than twice as high (8.3μmho/day) for anaerobically treated plants as for aerobically treated plants (4.0μmho/day). In the anaerobic treatment however, although the initial increase is smaller, there is a continued steady increase, indicating, as is beginning to become apparent that, after 14 days, the final total conductivity in this treatment would eventually surpass that in the aerobic treatment.

Conductivity in the media around Picea sitchensis roots remains at a low level, though again if this is related to root volume it is higher than is initially apparent. Conductivity attained in aerobic conditions is, as was observed for potassium loss, higher (89.25 μmho after 14 days) than that in anaerobic conditions (52.25 μmho after 14 days). In this case, conductivity in the aerobic treatment is higher than that in the anaerobic treatment throughout the experiment. The histograms show (fig. I/6), as was found in Picea sitchensis for potassium that increase in conductivity is also in fact maintained at a higher level in the aerobic rather than the anaerobic treatment found with Pinus contorta. The graphs also indicate that the rate of increase in conductivity is higher in aerobic conditions (3.9 μmho/day) from anaerobic (2.2 μmho/day) for Picea sitchensis.

PH = EXPERIMENTAL SERIES A: In both species, the pH increases in aerobic treatment and decreases in anaerobic (the initial pH being 6). For Pinus contorta the pH reaches pH 6.46 after 24 hours of aerobic treatment, and pH 5.49 after 24 hours of anaerobic treatment. For Picea sitchensis the highest pH attained in aerobic conditions is pH 6.47 after 12 hours, although this appears to fall slightly to pH 6.38 after 24 hours. Likewise, in anaerobic conditions, the lowest pH, 5.77, is reached after 12 hours, and then rises slightly to pH 5.81 after 24 hours.

PH - EXPERIMENTAL SERIES C: Similar results to those in experiment A are obtained. For Pinus contorta pH reaches pH 6.4 after 14 days aerobic treatment, and pH 5.5 after 14 days anaerobic treatment. For Picea sitchensis pH reaches a high 7.1 after 14 days of anaerobic treatment, and pH 5.56 after 14 days aerobic treatment. (fig. I/7).

TOTAL CARBOHYDRATES, GLUCOSE AND AMINO-ACIDS - Experimental Series A: In the first experiment samples of the root media were analysed for total carboydrates and amino-acids. However, although slight amounts of these substances were detected in some cases, the results were eratic, and it was considered that the experimental design may have been too "coarse" for satisfactory quantitative estimation of these substances.

2. B. Discussion

The results from the three experiments, two short term, and one of longer duration, show a general agreement. This emphasizes the validity of the observed effects, as the apparatus used in Exp. B was different (and hence the volume of liquid around the roots and the oxygen concentration), and the trees used in EXP. C were from different provenances to those used in EXP. A and EXP. B. All experiments confirm the fact that the subjection of the roots of Pinus contorta seedlings to anaerobic conditions, increases the leakage of certain substances from the roots. Picea sitchensis roots subjected to the same conditions display no such definite response, and in fact, leakage from roots in aerobic conditions is often greater.

Both species showed an increase in pH of the external medium under aerobic conditions, and a decrease under anaerobic conditions. Since the seedlings were in distilled water, the changes in pH are probably primarily due to the loss of solutes from the roots, rather than their uptake.

Increase in pH is normally associated with cation loss. The increase in pH shown in aerobic conditions is much more marked in Picea sitchensis than in Pinus contorta, Picea sitchensis showing an increase in pH of 1.1 above the initial level, whereas Pinus contorta showed a change of only pH 0.4 from the initial level of pH6. These observations can be related to the results for potassium leakage, where potassium leakage from Picea sitchensis roots in aerobic conditions was found to be higher than leakage from Pinus contorta roots in aerobic conditions. Despite the marked difference in potassium leakage for the two species in anaerobic conditions, the final pH after 14 days of anaerobiosis is remarkably similar, and is pH 1 to 1.5 below the initial pH6. This decrease in pH is presumably due to increased loss of organic acids and amino acids (Hiatt and Lowe 1967). Although these substances were not detected in these experiments, evidence for their excretion has been found by other workers (Grineva 1962; Hiatt and Lowe 1967; Katznelson et al 1955). However, in consideration of the different reactions to anaerobiosis shown by the two species as demonstrated by the results of potassium loss and conductivity, the similarity in pH shown here is remarkable.

The meaning of the results obtained in this section must be related to the possible causes of leakage of substances from plant roots during anaerobiosis, some or all of which may be operative.

Livingstone and Free (1917) state that the first effect of oxygen deprivation is an interference with the absorption of water by the roots. This effect may have some bearing on the mechanism of leakage. The very fact that it occurs means also, that there will be a reduced uptake of oxygen dissolved in the water, thus aggravating the condition. Although a recognised symptom of flooding damage (Hagan 1950; Kramer 1951; Kramer and Jackson 1954; Letey, Stolzy and Blank 1962; Yamasaki 1952), the reasons for this reduction in water absorption are not completely clear. Kramer and Jackson (1954), when analysing

the changes in water permeability of flooded tobacco plant roots, found there was not a simple decrease in water absorption on flooding, but a decrease, followed by an increase, a further decrease, and then possibly another increase. They attribute the initial decrease in water permeability of the roots to water, to an effect of the increased viscosity of the protoplasm. Low O_2 and high CO_2 levels stop cyclosis and increase the viscosity of protoplasm (Seifriz 1945). The initial decrease in water absorption is followed by the death of cells, which causes some increase in water permeability, but is followed by the plugging of the xylem with dead material, resulting in a marked decrease in water absorption yet again. Finally, there may be an increased water permeability arising from the decay of the root system. Grable (1966) adds to this the fact that the roots of many deficiently aerated plants increase in diameter (Hook, Brown and Kormanik 1971; Kramer 1951b; Waddington and Baker 1965) which increases the distance for mass flow of water into the stele. It is also observed that anaerobic conditions supress the growth of root hairs (Daubenmire 1959 cited by Grable 1966; Yu, Stolzy and Letey 1969) or cause their lignification (Yamasaki 1952), and also limits water uptake in the longer term.

Letey et al (1962) connected reduced removal of water from the soil in flooded conditions, with reduced transpiration. However, Erickson (1965) has shown that conditions which stimulate transpiration (e.g. high light intensity) aggravate the symptoms of water loss in flooded plants. This would indicate that the reduction in water absorption is not a consequence of reduced transpiration (i.e. an interference with the driving force), but of increased resistance to movement through the roots. Hagan (1950), considered that this increased resistance was not due simply to the decrease in absorbing surfaces resulting from the death of roots, but was due to a direct effect of deficient aeration on cellular activities which influence water permeability. Livingstone and Free (1917) carried out experiments using root atmospheres of various composition, and found that the plants were sensitive to the supply of oxygen

per unit of root surface. This led them too, to suggest that the cause of reduced water absorption was direct, and due to interference with the respiration of the protoplasm of the root cells, which resulted in the death of the protoplasm, and the "consequent failure of the roots to function as water absorbers for the plant". However, they did not, as this may imply, put forward water absorption as an active process, but simply suggest that the coagulation of the dead protoplasm provided a physical barrier to water absorption. This is of course equivalent to Kramer's conception of "plugging".

It is Grineva (1962) who links this widely observed phenomenon of reduced water absorption, with leakage. She suggests that "leakage" is active excretion rather than passive leakage. This occurs to maintain the osmotic balance upset by the reduced water intake. Hence, the greater the effect that flooding, or deficient aeration has on reducing water uptake, the greater would be the level of exudation.

However, a large number of workers, (Haitt and Lowe 1957; McCullough and Simon 1973; Simon 1974) have considered that the primary cause of leakage in anaerobic, and certain other stress conditions, is due to the reduced energy output experienced during anaerobic conditions. This is because anaerobic respiration produces only 2 moles of ATP from one mole of glucose, as opposed to the 36 moles of ATP from one mole of glucose, obtained from aerobic respiration. This level of ATP production is said to be insufficient to maintain membrane structure. This includes both the repair of structural damage (Stewart and Guinn 1969) and also the continuing active turnover of phospholipids (Simon 1974). McCullough and Simon (1973) found that during inhibition of respiration (in this case by iodoacetate) there was a gradual reduction in phospholipid levels, the phospholipid which disappears most rapidly being that with the most rapid turnover rate. Fulton, Erickson and Tolbert (1964) also found that flooding the soil decreased the percentage of photosynthetically

fixed C^{14} in the lipid fraction of tomato plants. Vartapetian, Andreeva & Kozlova (1976) described the disintegration of the mitochondria of rice seedlings during anaerobic treatment, and attributed this to the lack of storage substances, which results in a lack of ATP regeneration, required to maintain these structures. However, since they found that addition of glucose did not prevent the disintegration of organelles in excised roots, it seems likely that even if ample storage products were available, the energy obtained by anaerobic breakdown would still be insufficient to maintain the membrane integrity of the mitochondria. Casteels et al (1972), when considering the effect of metabolic depletion on the membrane permeability of smooth muscle cells, found that there was an increase in permeability (to sorbitol) and a decrease in ATP during N_2 treatment. They too state that the ATP levels measured are insufficient to maintain normal membrane structure. However, they pose the question - is the decrease in ATP a direct cause of the membrane modification, or, is decrease in ATP and increase in permeability two independant phenomena caused by the disturbance in cell metabolism?

Grineva (1963), in her work on sunflower and corn roots, considers that enhanced consumption of sugars and an increased rate of glycolysis in anaerobic conditions is adaptive, since more ATP is produced in these circumstances. However, an increased rate of glycolysis in anaerobic conditions may not in fact be advantageous. Crawford and McManmon (1968) found that when plants were treated anaerobically, the ethanol resulting from anaerobic respiration caused the induction of the enzyme ADH. It was also found that ADH induction was far greater in those species intolerant of flooding. Marshall et al (1973) traced the difference in tolerance of flooding in two strains of maize, to a difference in one gene. They found that maize plants homozygous for the slow activity allele for ADH were better fitted for survival in flooded conditions, than those plants homozygous for the more active ADH allele. In both these cases, the increase in ADH would lead to an increased rate of glycolysis,

increased consumption of sugars and increased ATP production. However, the increased glycolysis also results in increased production of ethanol, and it seems that the detrimental effects of this toxic end product, override the beneficial effects of increased energy production.

It has been suggested that one of the effects of ethanol may be to actually start dissolving cell membranes via its solvent action on lipids (Crawford (1977)); Huck and Erickson unpublished. This action would be expected to have the effect of decreasing the semi-permeability of the membrane, and increasing leakage. Gudjonsdotter (1962) noted that the primary alcohols were more toxic to wheat roots than the secondary and tertiary alcohols tested, and suggested that toxicity may be related to degree of lipophilly and subsequent membrane damage. The "fluidising" effects of alcohols on all membranes have also been noted in connection with observations on their properties as anaesthetics (Kiyosawa 1975; Schnedier 1968; Seeman, Sha'afi, Galey & Solomon 1970; Seeman, Roth and Schneider 1971). This work has suggested that during anaesthaesia, alcohol molecules become bound to non-polar sites in the plasma membrane and because of this, may increase or decrease permeability, according to their concentration (Kiyosawa 1975). At low concentrations, the alcohol molecules interact with the membrane to make the equivalent pore radius of the membrane narrower, but at higher concentrations, this interaction becomes great enough to disrupt the membrane and cause loss of semi-permeability.

It is also possible that, as leakage continues due to one or some of the above causes, the resulting increase in acidity of the root medium may play a secondary role in increasing leakage (Christiansen, Carns and Slyter 1970).

Is it possible from the results of this experiment to throw any light on the processes operating during leakage? Clearly more information is needed before any deductions may be made, and an attempt to gather some of this is presented in Chapter IV. It can be said that, in accordance with published observations

on other species, the roots of Pinus contorta do appear to "leak" more in anaerobic conditions. However, it is interesting to speculate why the roots of Picea sitchensis appear to "leak" more in aerobic rather than in anaerobic conditions. This is apparently a real result, since it is confirmed in both the experiments (EXP. A and EXP. C) where leakage in aerobic and anaerobic conditions are compared. It is also shown both in the rates of increase in conductivity and potassium levels. At this point, the only theory of those discussed which fits with this data in any way, is that which considers leakage to be an active process. Picea sitchensis is sensitive to flooding (Boggie 1974; Vester 1972) and may be affected by even aerated water (Kramer 1965), thus suffering reduced water uptake in both treatments. Possibly there was only sufficient oxygen to provide for active exudation in the aerobic treatment. However, since Picea sitchensis is particularly sensitive to flooded conditions, it would have been more likely that the levels of exudation, or leakage, would be higher in both treatments, as it is probable that passive leakage plays a much more major role in leakage than active exudation. Exudation is generally observed to increase in anaerobic conditions, therefore it is unlikely that it is primarily an active process. An alternative explanation is that roots are highly sensitive to the mechanical effect of forced aeration, (Hoagland & Arnon 1938) and suffered root damage because of this.

This method of observing the effects of reduced aeration on root systems has no application as a field indicator of flooded conditions. Apart from the obvious fact that it cannot be easily translated into a field method, these results show that, in water culture at least, leakage may be increased or decreased in anaerobic conditions according to the species.

3. EFFECT OF ROOT ANOXIA ON CHLOROPHYLL LEVELS

As indicated in the introduction, chlorosis of the leaves of plants subjected to flooding, is a phenomenon which has been frequently observed. The chlorophyll contents of needles of Pinus contorta and Picea sitchensis were determined in one short and one longer term experiment (EXPERIMENTAL SERIES B and EXPERIMENTAL SERIES C).

3. A. Results

Experimental Series B: Results from this experiment are far from satisfactory. There is great variation between the chlorophyll levels of different plants, and no trend of chlorophyll levels is distinguishable after 8 days (fig. I/8).

Experimental Series C: In this experiment, samples were taken more frequently, and consisted of a greater number of needles. Also this experiment was conducted with roots in sealed bottles rather than open pots as used in EXPERIMENT B, thus oxygen content of the water was much lower (figs. I/1 & I/2).

The overall trend shown by these results are a reduction in chlorophyll levels over 14 days in Picea sitchensis in both aerobic and anaerobic conditions, and in Pinus contorta, an initial fall in the level of chlorophyll followed by a rise back to original levels, both in aerobic and anaerobic conditions. The levels of chlorophylls a and b follow the same trends as total chlorophyll (fig. I/9). Anaerobic treatment does not appear to have a marked effect on the chlorophyll levels of either of these species, and, in fact, aerobic conditions appeared to have a more detrimental effect on chlorophyll levels in Picea sitchensis, than does anaerobic conditions. However, in this case, the overall levels of chlorophyll after 14 days are lower in Picea sitchensis (maximum of 9.93 mg/mm^2), than they are in Pinus contorta (maximum 16.99 mg/mm^2) (fig. I/8).

Fig. I/8

Chlorophyll content of needles from tree seedlings in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels (Experimental Series C); or in aerated or unaerated open 2 litre jars (Experimental Series B).

▲ Pinus contorta Experimental Series C

△ Pinus contorta Experimental Series B

● Picea sitchensis Experimental Series C

○ Picea sitchensis Experimental Series B

– – – aerobic
——— anaerobic
} in each case

Each point represents the mean value for three (Experimental Series B) or two (Experimental Series C) culture vessels.

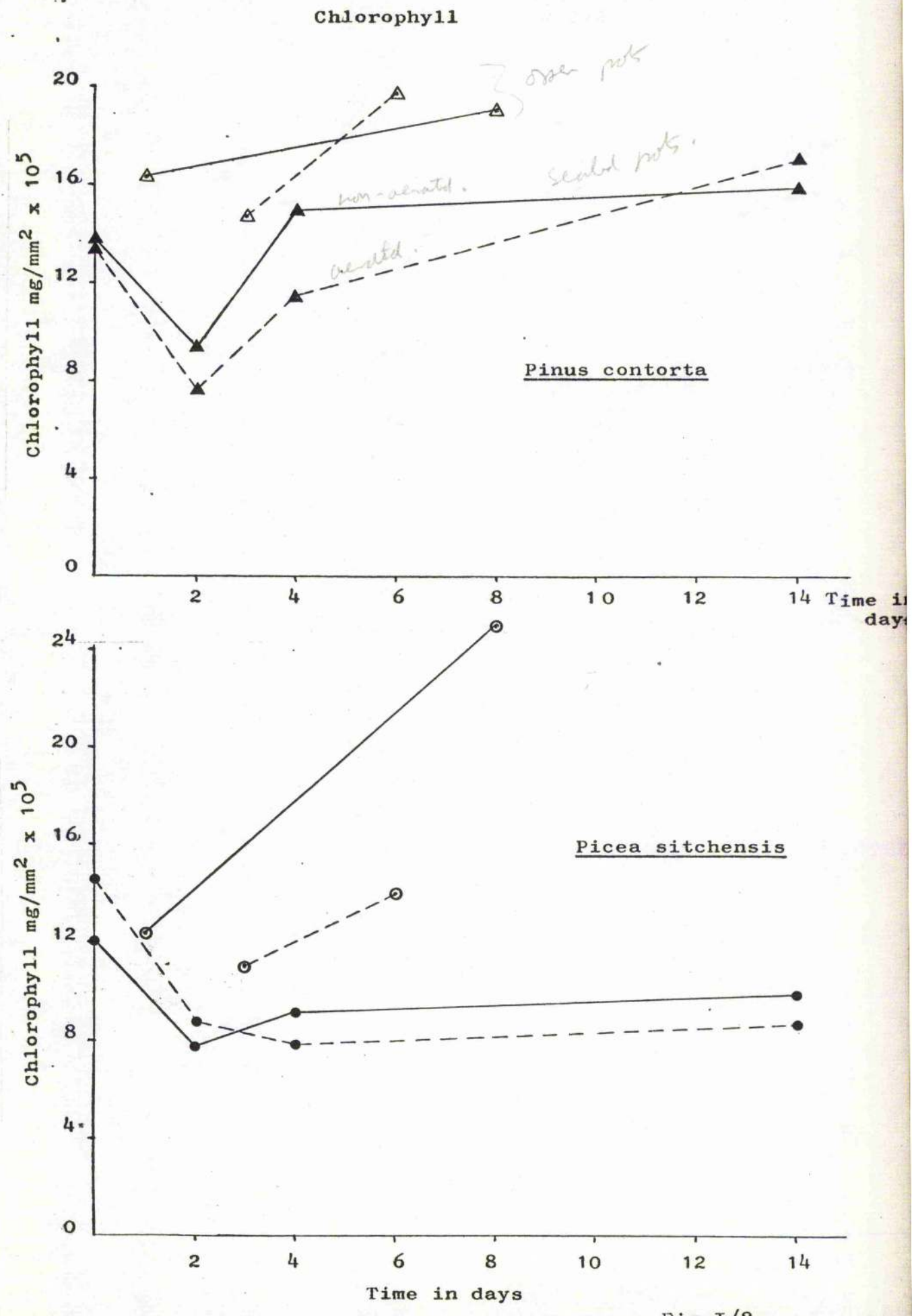

Chlorophyll
Chlorophyll mg/mm² x 10⁵
20
16
12
8
4
0
non-aerated.
aerated.
Pinus contorta
2
4
6
8
10
12
14
Time in days
24
20
16
12
8
4
0
Picea sitchensis
2
4
6
8
10
12
14
Time in days

Fig.I/8

Fig. I/9

Chlorophyll a and b content (mg/mm^2) of leaves from seedlings of Pinus contorta and Picea stichensis in water culture in sealed (anaerobic) or unsealed and aerated (aerobic) 600 ml vessels.

Experimental Series C.

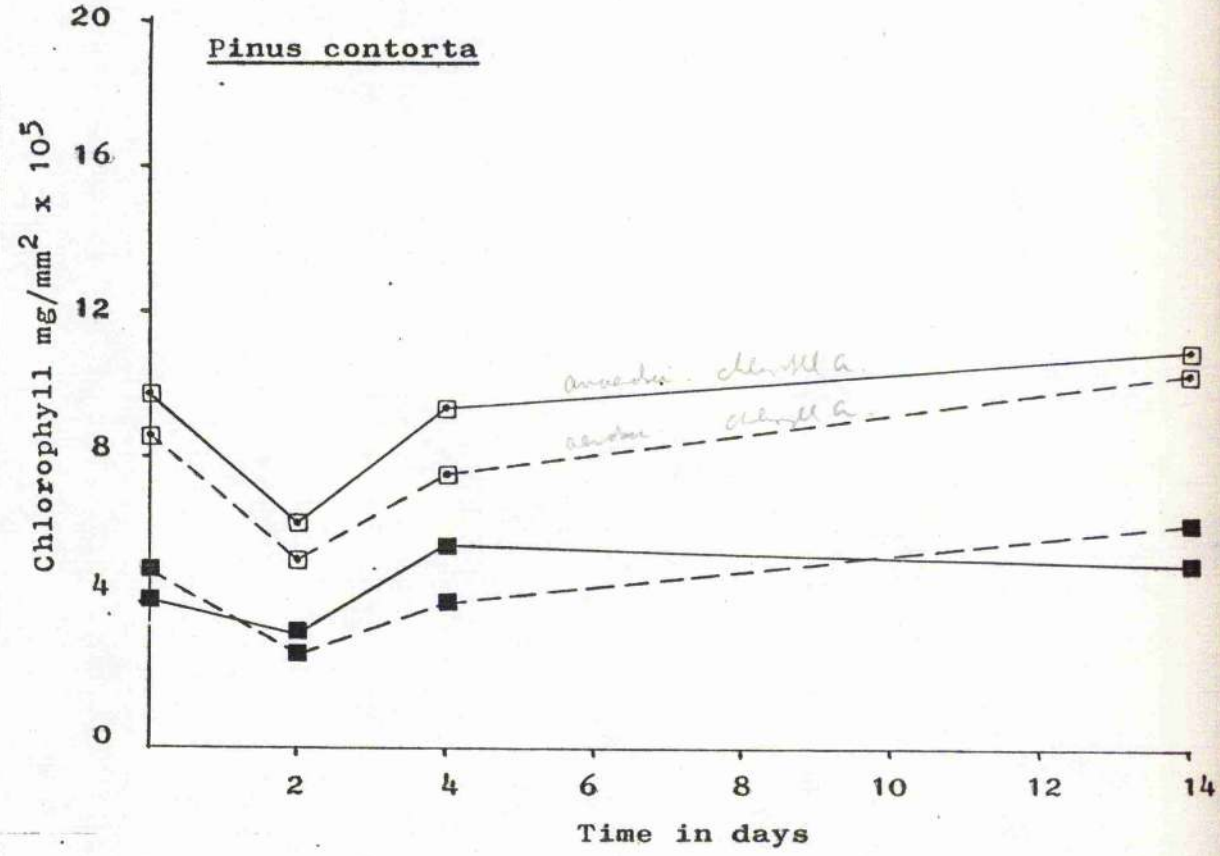
□ Chlorophyll a

■ Chlorophyll b

- - - aerobic
——— anaerobic
} in each case

Each point represents the mean from two culture vessels.

Chlorophylls a and b



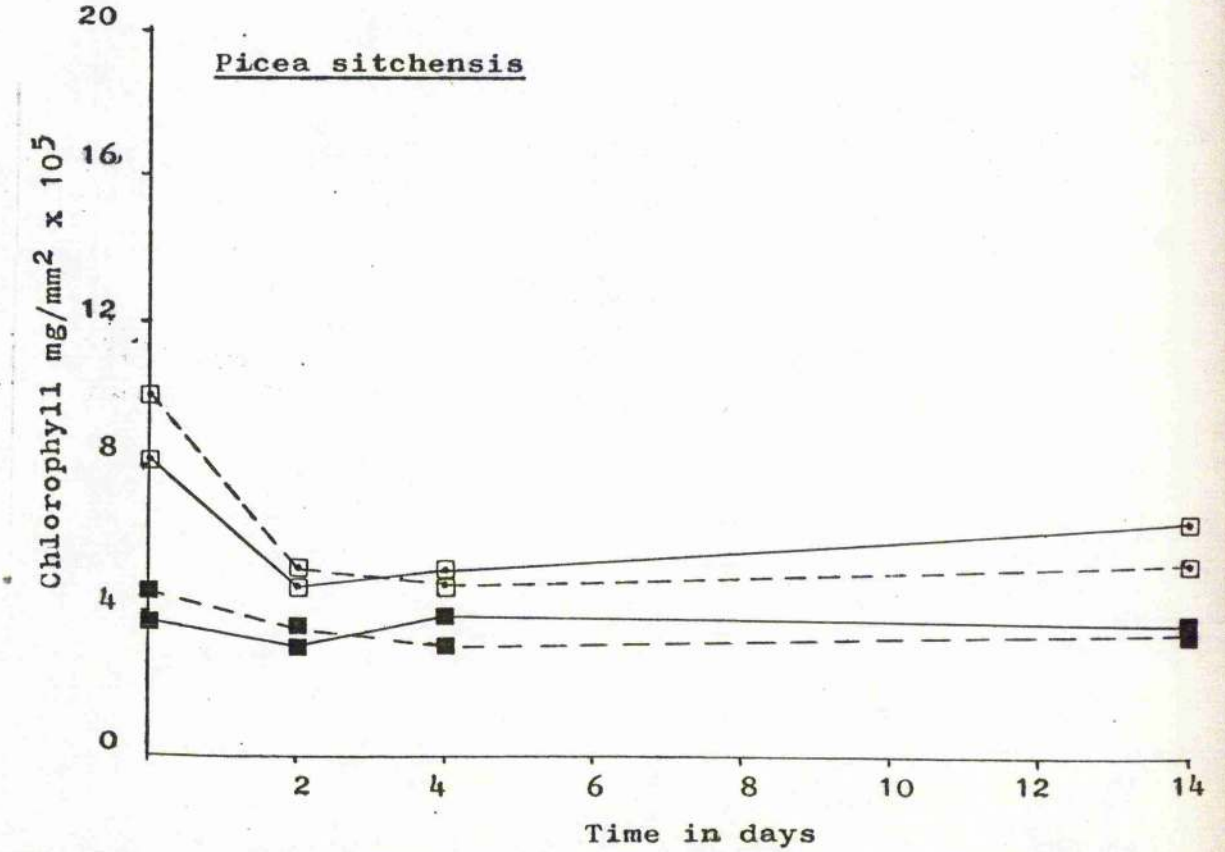


Fig.I/9

3. B. Discussion

No real conclusions can be drawn from the results of this experiment other than to reiterate the view expressed in the introduction to this chapter, that the measurement of the effect of anaerobic conditions on the level of chlorophyll is difficult to measure especially using small samples of leaves over short periods of time. The great variability of chlorophyll content of needles from different trees, makes it difficult to ensure valid results. However it is interesting to note the repeat of the trend found in the preceding section, that is that aerated water culture appears to have a more detrimental effect on Picea sitchensis than unaerated water culture. The reasons for this are hard to establish, but presumably if the roots are being damaged by the physical action of the aeration, this would be reflected in the leaf chlorophyll levels, in a similar manner to the damage caused by anaerobic conditions.

4. ETHANOL PRODUCTION

The production of ethanol and carbon dioxide by higher plants in anaerobic conditions was first demonstrated by Le Chartier and Bellamy (1869). Ethanol is a substance of wide occurrence and distribution among plants and organs of plants, but wherever it is found, its presence is exclusively as a result of the blockage of the electron transport system. When there is a lack of oxygen an alternative electron acceptor must be found to "store" the oxygen debt. This acceptor may eventually be "repaid", stored, or excreted. Unless such an acceptor is found in the absence of oxygen, all energy production would cease and the cells would die. The production of ethanol as an end product of anaerobic respiration is one method of storing this oxygen debt, and ensures a continued, though reduced, energy production from the glycolitic cycle, and the regeneration of NAD, which is required earlier in the cycle. Oxygen debt may also be stored in other compounds such as lactate, glycerol, malate (see fig. I/10) or amino acids, but

ethanol is by far the most ubiquitous in higher plants. Reports of the occurrence of ethanol have come from roots (Crawford 1967), seeds (Leach 1936), Tubers (Barker and Safti 1952), fruits (Thomas 1931) - any organs where an anaerobic regime may prevail.

The ability to respire anaerobically is of considerable ecological importance, since without it, survival of even brief periods of anaerobic conditions, such as temporary flooding after heavy rain, would not be possible. Plant roots in these circumstances would literally die of suffocation. The ability of plants, crop plants in particular, to grow in flooded, or badly drained soil, is also, of course, of considerable economic importance.

The higher ethanol content of plant tissues respiring anaerobically has been demonstrated several times (e.g. Crawford 1966; Hageman and Flesher 1960). Since the imposition of an anaerobic root regime is the principle effect of flooding the soil, it is possible that the degree of oxygen deficiency may be reflected in the amount of ethanol produced as a result of anaerobic respiration in the roots. To investigate this possibility, samples were taken from trees of Pinus sylvestris from an area of Tentsmuir Forest (NO. 490260) consisting of dunes and slacks.

4. A. Results

The results are expressed in the form of histograms (fig. I/11). These show that, in all cases, readily detectable amounts of ethanol are present in the wood core samples. This indicates that a certain degree of anaerobic respiration was in process in the roots of the trees from all sites, including the driest. However, the main significance of these results are that they show clearly that those trees from the wettest areas, that is, the wettest part of the slacks, produce the greatest amount of ethanol. The highest amount of ethanol recorded here (10.67 μM/g dry wt.), is more than 7 times even the highest value for ethanol recorded from trees in the dry dunes (1.41 μM/g dry wt.). Levels of ethanol are also seen to be correlated to some extent, with the wettest and driest regions within each area.

Fig. I/10

Alternative storers of "oxygen debt"

Summary diagram illustrating the various means of proton disposal and the range of end products of glycolysis found in plants capable of enduring prolonged periods of partial or total anoxia.

(Crawford 1976).

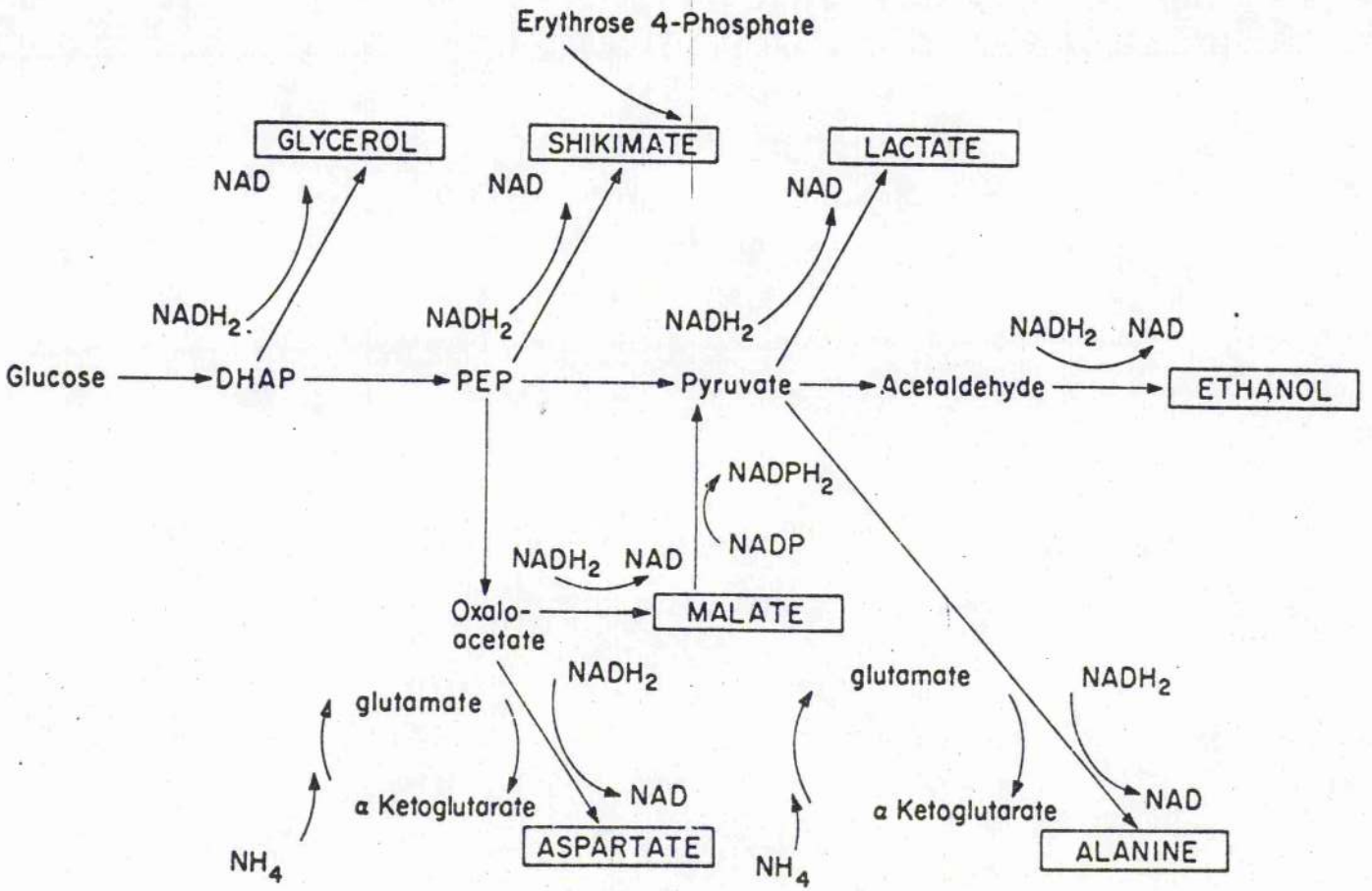

Erythrose 4-Phosphate
GLYCEROL
SHIKIMATE
LACTATE
NAD
NAD
NAD
NADH2
NADH2
NADH2
NADH2 NAD
Glucose
DHAP
PEP
Pyruvate
Acetaldehyde
ETHANOL
NADPH2
NADP
NADH2 NAD
Oxalo-
acetate
MALATE
NADH2
glutamate
NAD
α Ketoglutarate
ASPARTATE
NH4
glutamate
NADH2
NAD
α Ketoglutarate
ALANINE
NH4

Fig. I/10

Fig. I/11

Ethanol content (μM ethanol/g dry wt.) of wood cores from ten trees of Pinus sylvestris growing in dunes and slacks in Tentsmuir, Fife.

Each column represents the mean value of separate ethanol determinations from two cores from each tree.

Ethanol content of wood cores taken from ten trees of Pinus sylvestris growing in dunes and slacks in Tentsmuir forest, Fife.
(October)

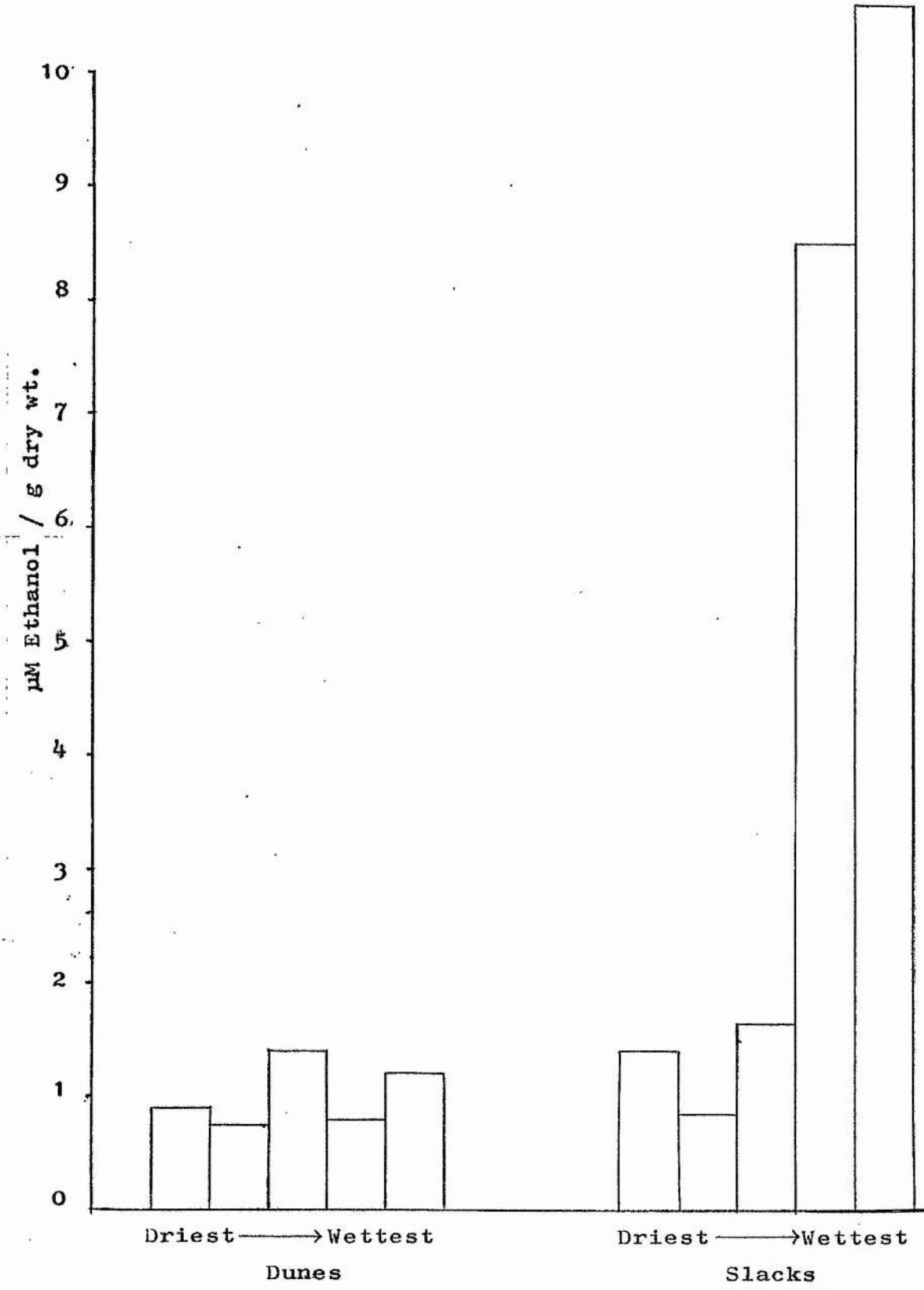


Fig.I/11

4. B. Discussion

The damage caused to many crop plants (Sartoris and Belcher 1949; Hoveland and Webster 1965) by periods of flooding is of great economic importance and has been the subject of a number of investigations (Erickson and Van Doren 1960; Letey, Stolzy and Blank 1962; Van't Woudt et al 1957). Considering this, and the occurrence of ethanol under these specific conditions, it is perhaps surprising that its use as a possible quantitative test for anaerobic conditions, relative tissue sensitivity, and tissue damage has not already been fully tried and tested.

In vitro studies, using incubated roots from flooded and unflooded plants have shown that root segments from flooded plants produce more ethanol than those from unflooded plants (Crawford 1966; Hook, Brown and Kormanik 1971). However, Aubertin et al (1966) reported no correlation between oxygen level and ethanol content of tomato and pea plant exudates in prolonged experiments, although Bolton and Erickson (1970) are of the opinion that this was due to their experimental procedure. Kenefick (1962) also found that higher ethanol levels did not always relate to duration of flooding in sugar beet. Aubertin (1966) had found that ethanol level was more closely related to morphological age of the plant used. Aubertin (1966) and Fulton and Erickson (1964) found that ethanol concentration was lower in prebloom as opposed to flowering plants of pea and tomato. This is consistent with observations (Erickson 1965; Erickson and Van Doren 1960; Erickson, Fulton and Brandt 1964; Van't Woudt and Hagan 1957) that peas and cereals are much more sensitive to oxygen deficiency just prior to blooming. Potatoes can be damaged by just one day of flooding if it is soon after planting (Erickson 1965), and low oxygen availability was most detrimental to sunflowers, cotton, and green beans in their early stages of growth (Letey, Stolzy and Blank 1962).

Whilst considering ethanol, other metabolites have also been suggested for use as indicators of anaerobic conditions, for example, γ-amino butyric acid (GAB) (Huck and Erickson unpublished; Erickson, Fulton and Brandt 1964; Fulton, Erickson and Tolbert 1964). In examining the feasibility of using this substance, the authors point out that, whilst the level of GAB does indeed increase in anaerobic conditions, its level is also greatly affected by other environmental stress factors, such as low light intensity or low nutrient level. Ethanol is also subject to some of these conditions, for example, it has been found to be higher in conditions of high light intensity (Fulton and Erickson 1964). However, it seems that, despite this, ethanol is still far less susceptible to fluctuations in the environment than GAB, (Huck and Erickson (unpublished); Erickson, Fulton and Tolbert 1964). These authors therefore suggest the measurement of ethanol for development as an assay for soil oxygen status. Fulton and Erickson (1964) did in fact manage to develop a quantitative assay for ethanol in tomato exudates, for flooding periods up to 12 hours. In this paper they state that the critical oxygen diffusion rate (CDR) for ethanol accumulation should be the same for all soils, although Greenwood (1968) has stated that, as far as growth is concerned, CDR, gas filled pore space, and oxygen content are not related to yield in the same way in more than a few soils. Huck and Erickson (unpublished) later, in fact, stress the need for knowledge of soil type, soil microflora, climate and specific crop/soil combinations. This is certainly true, since there is now increasing evidence (Crawford 1966; Chirkova, Khazova and Astafurova 1974; Chirkova 1975) that some species, subspecies or even ecotypes, differ in their ethanol production and in their tolerance to flooding.

However, even this limited survey has shown that comparative measurements of ethanol from trees of the same species in areas differing in water table levels, can give some indication of where the water table is highest and/or is having the greatest effect on the roots of the trees. If such an simple measurement can in some way be calibrated to give such information, it would be a particularly useful

and important tool for the forestry industry. Obviously many variables concerning both the plant material and the physical conditions of the site would have to be taken into account, but the fact that such promising results can be obtained from such a brief survey as this outlined here, make it an exciting possibility for the future, warranting further investigation.

5. GENERAL CONCLUSIONS

The investigations in this chapter have shown that analysis of ethanol from the roots of plants in flooded conditions may be a useful and ongoing method to measure flooding of the soil and flooding damage in the plant. It can also be developed to involve a simple field sample collection procedure.

The parameters of root leakage and leaf chlorophyll content, whilst they may give some information as to the type of processes which are occurring during flooding damage, are of no practical use as indicators of this condition. The high levels of ethanol found in flooded trees may provide a significant clue as to the nature of flooding damage.

CHAPTER II

SEASONAL FLUCTUATIONS OF ETHANOL LEVELS IN WOOD CORES OF Picea abies and Pinus sylvestris

1. INTRODUCTION

In the last chapter, section 4 showed that ethanol may prove to be a useful indicator of flooded conditions in the field. For this reason, it was decided to carry out a more extensive survey at Tentsmuir Forest, Fife, using three sites varying in proness to flooding. Tentsmuir Forest is situated on an area of newly acreted soil from a former dune and slack system, and it has been shown (Crawford and Wishart 1966), that the movements of the water table here can be easily measured and predicted. The first site chosen was on a former sand dune (site NO. 48 map ref. NO 498241, fig. II/1) and trees here were never flooded, whilst those on the second site, an ancient slack (site NO 36 map ref. NO 497248, fig. II/1) were subject to the rise and fall of a water table, usually 1-2 metres below the soil surface. The third site (NO. 129 map ref. NO. 463266, fig. II/1) was by the side of a loch and was regularly flooded in the winter. The trees on the first two sites were Pinus sylvestris and on the third site they were Picea abies.

Initially, samples were collected both from the roots and from the base of trunks (using a tree borer). Two sets of cores were taken, one set being placed directly into perchloric acid in McCartney bottles, and the other set being placed into empty dry McCartney bottles. (All sets were later extracted in perchloric acid). These three sets of samples were taken so that results of each could be compared. This was to ascertain whether the last mentioned method, that is, the simplest, would suffice, since the aim was to find the simplest method of field collection which could be used to attain equally informative results. As a result of this comparison, the remainder of the study was continued by collecting wood core samples directly into dry McCartney bottles. The samples were collected at approximately monthly intervals over a period of a year. (Maps of Tentsmuir - figs. II/1 & II/2).

Fig. II/1

Tentsmuir Forest, Fife, showing drainage and depth of water table. Site numbers are the upper numbers in each pair. Sites where samples of wood cores were taken, are arrowed (sites 36, 48 and 129.).

Tentsmuir Forest, Fife

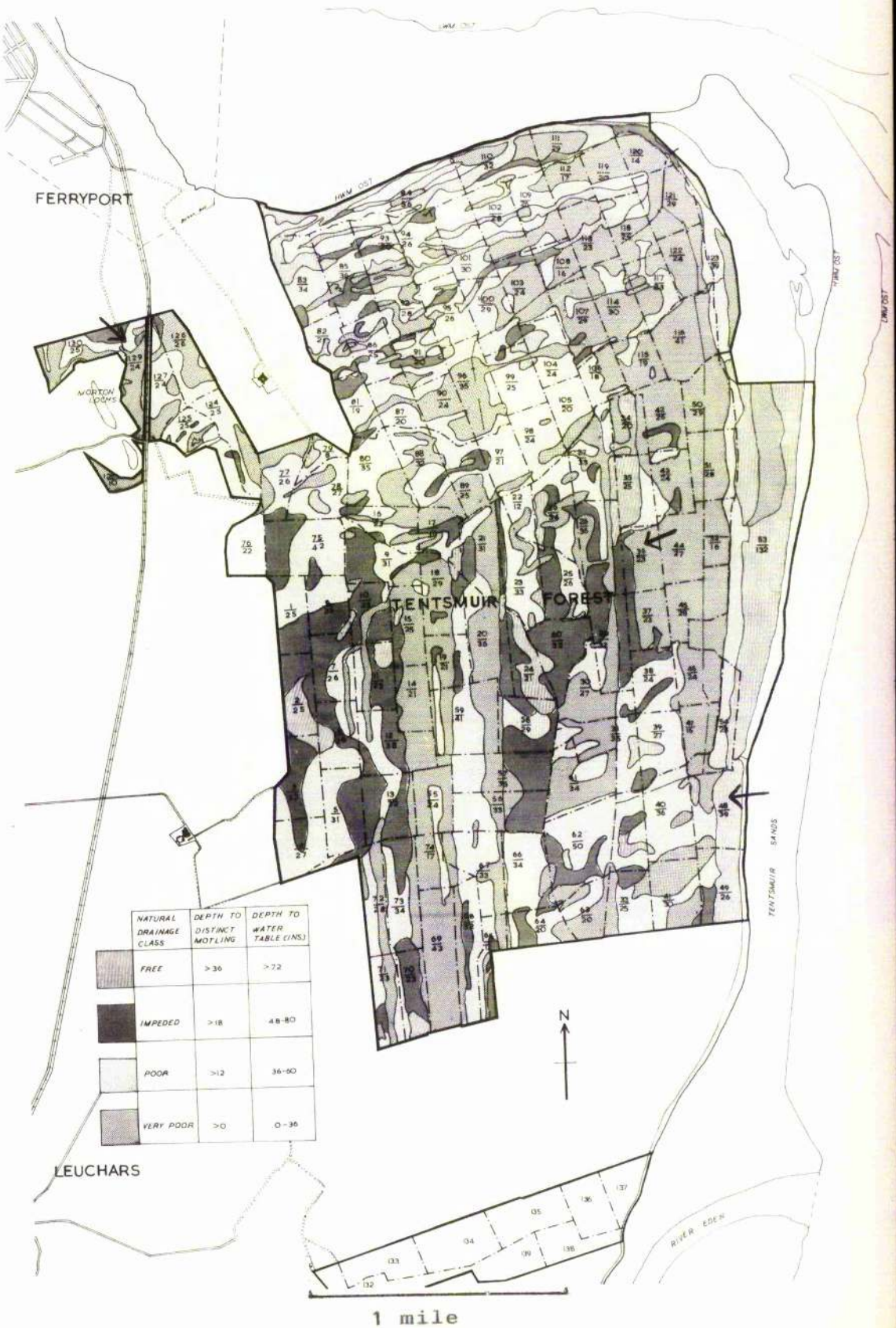


Fig.II/1

Fig. II/2

Tentsmuir Forest, Fife, showing tree species grown.

Key:

SP	—	Scots Pine (Pinus sylvestris)
CP	—	Corsican Pine (Pinus nigra)
LP	—	Lodgepole Pine (Pinus contorta)
NS	—	Norway Spruce (Picea abies)
SS	—	Sitka Spruce (Picea sitchensis)

(The number following 'P' on the map indicates the year of planting)

XXX — Trackways

Tentsmuir Forest, Fife

Fig.II/2

Fig. II/3

Correlation between ethanol levels in trunk wood cores, and root samples, of Pinus sylvestris and Picea abies. (Wood core samples collected directly into 6% perchloric acid)

Correlation (r) = 0.83

P = 0.01

Each plot represents the comparison between a wood core and a root sample collected from the same tree.

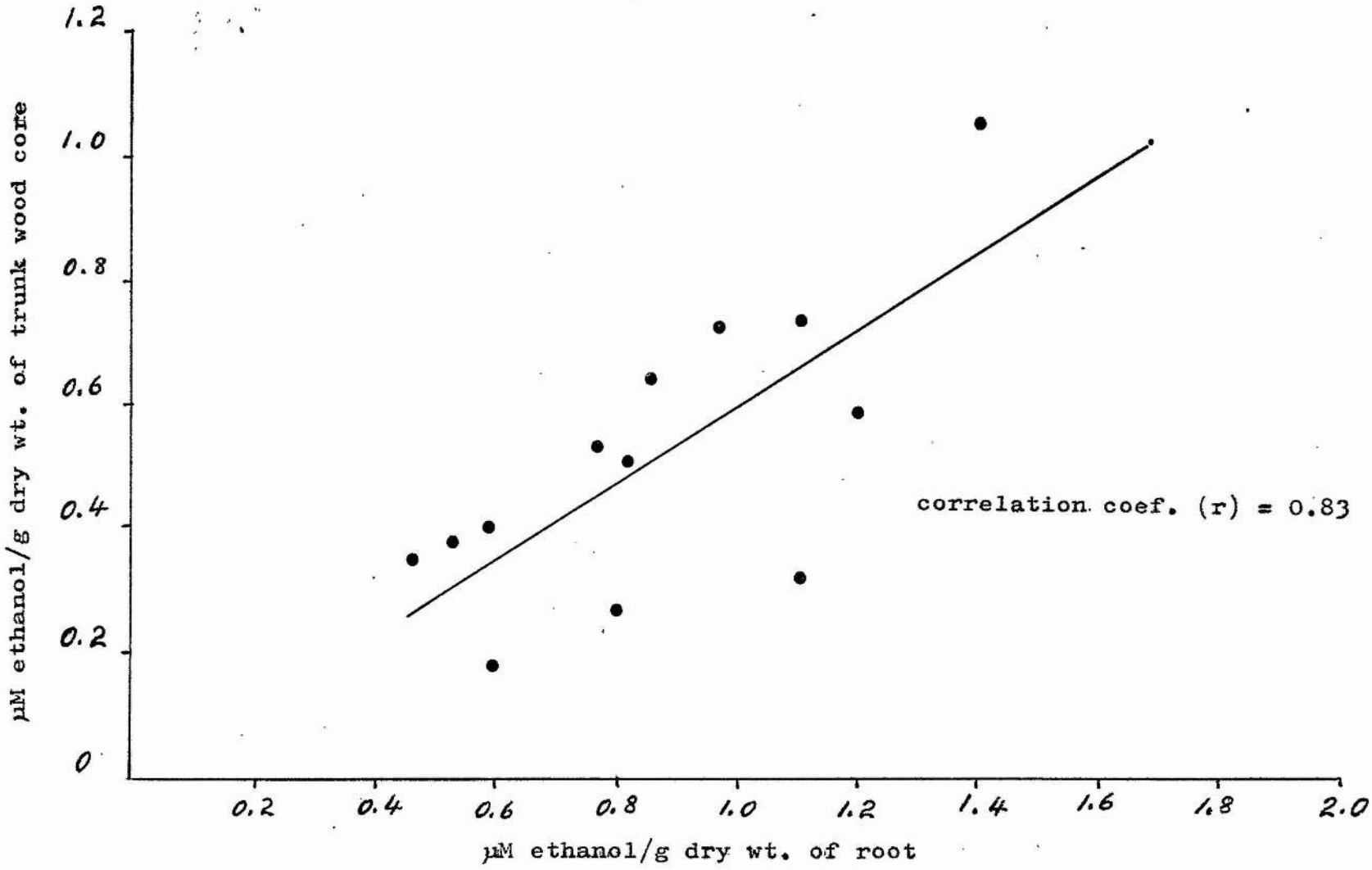

1.2
1.0
0.8
0.6
0.4
0.2
0
µM ethanol/g dry wt. of trunk wood core
correlation coef. (r) = 0.83
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
µM ethanol/g dry wt. of root

Fig.II/3

Fig. II/4

Correlation between ethanol levels in wood cores of Pinus sylvestris and Picea abies collected either "dry" in an airtight container, or directly into 6% perchloric acid in a similar container.

Each plot represents the comparison between two cores collected from the same tree.

Correlation coefficient (r) = 0.84

P = 0.01

Ethanol content of wood-cores from
Tentsmuir, Fife

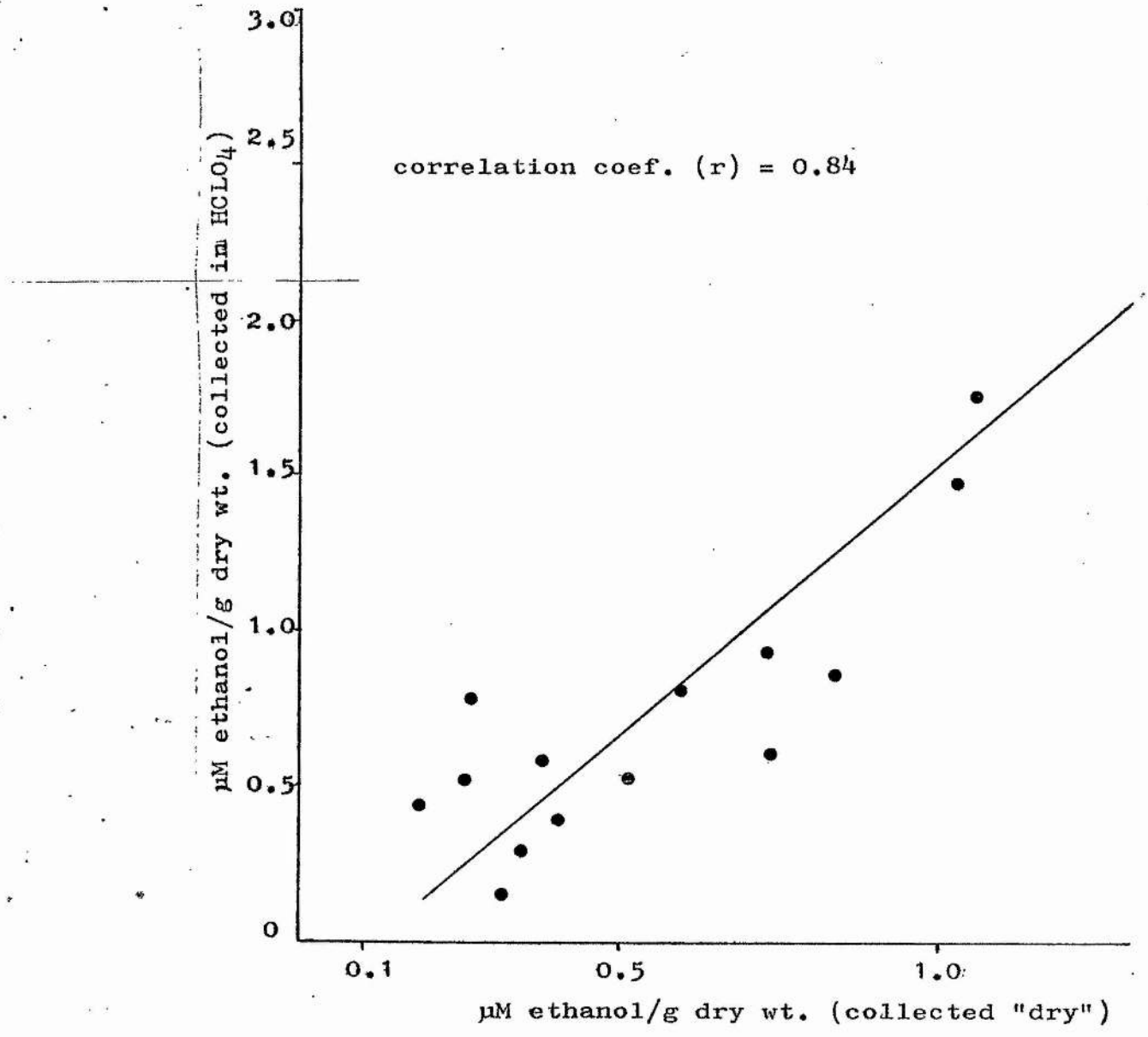


Fig.II/4

Fig. II/5

Monthly levels of ethanol (μM ethanol/g dry wt. of wood core), detected in wood cores from the base of trunks of mature trees of Picea abies and Pinus sylvestris growing at Tentsmuir, Fife.

- ● Picea abies growing in wet site with prolonged winter flooding, Morton lochs (site 129)
- ▲ Pinus sylvestris growing on a former dune slack (site 36)
- △ Pinus sylvestris growing on an ancient sand dune, Kinshaldy (site 48)

Error bars are based on ten separate estimates using two samples from each of five trees.

Ethanol detected in wood cores from Tentsmuir throughout the year

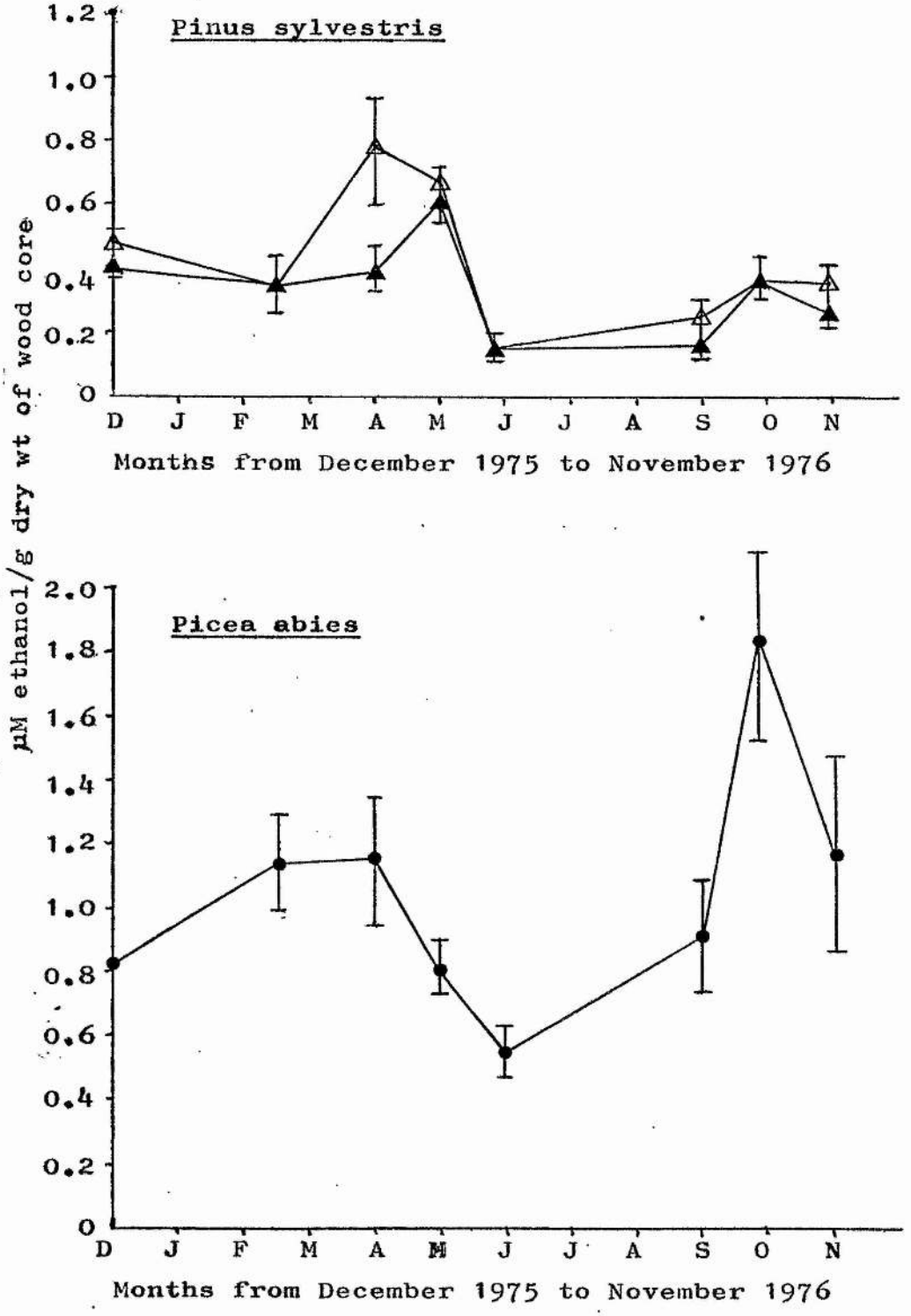


Fig. II/5

Fig. II/6

Graphs of a) Temperature C, b) Rainfall (cms) and depth of water table below the soil surface (cms), at Tentsmuir, Fife.

Temperature C°, rainfall (cms) and depth of water table (cms) throughout the year at Tentsmuir, Fife.

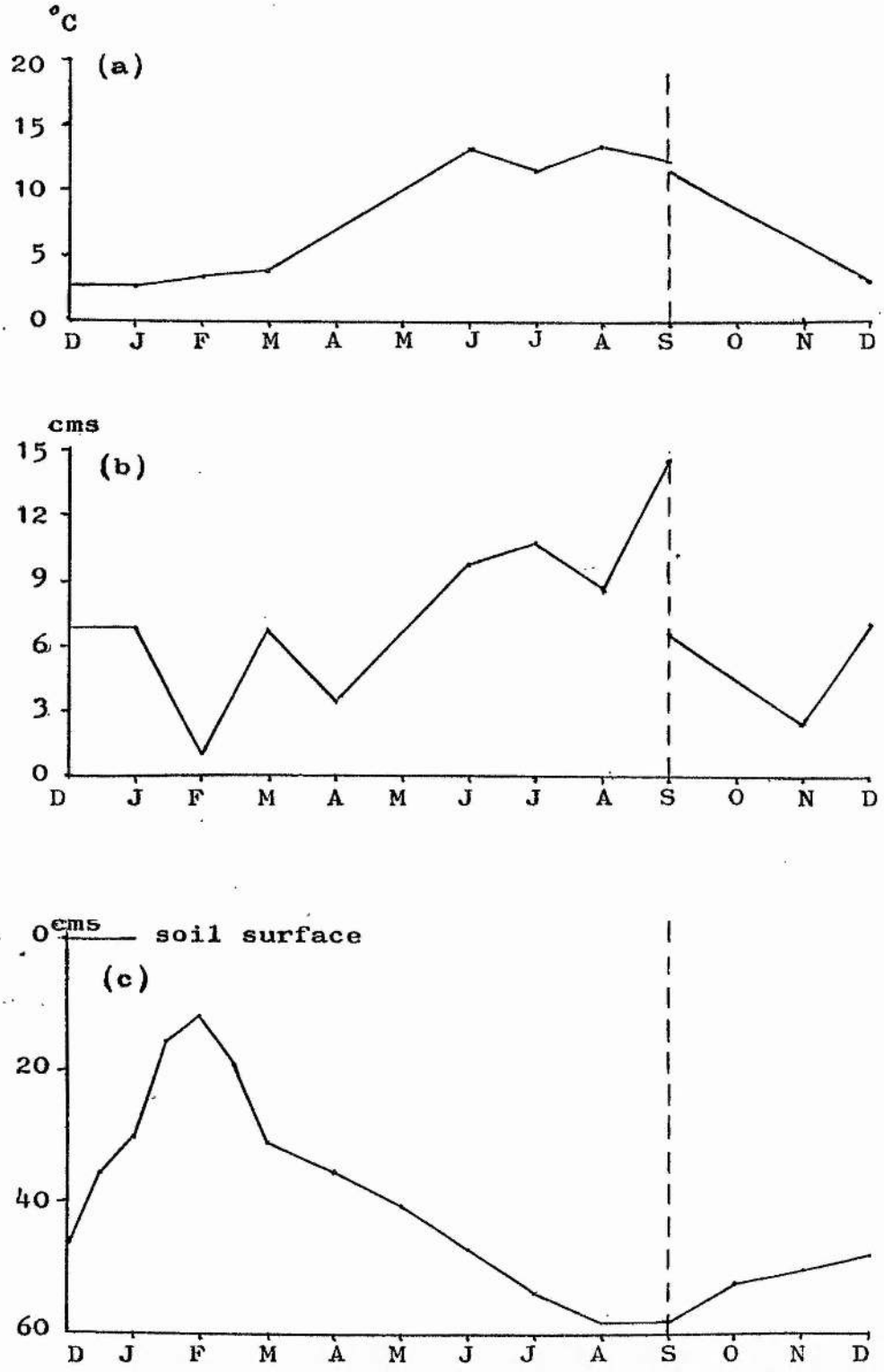


Fig.II/6

Fig. II/7

Monthly levels of ethanol (μM ethanol/g dry wt), detected in wood cores from the base of trunks of mature trees of Picea abies growing at Morton lochs (site 129) Tentsmuir, Fife.

Each graph represents the levels of ethanol detected in each of five individual trees. Each point is based on the mean of two estimates found using two core samples from each tree.

Vertical bars show maximum and minimum values recorded.

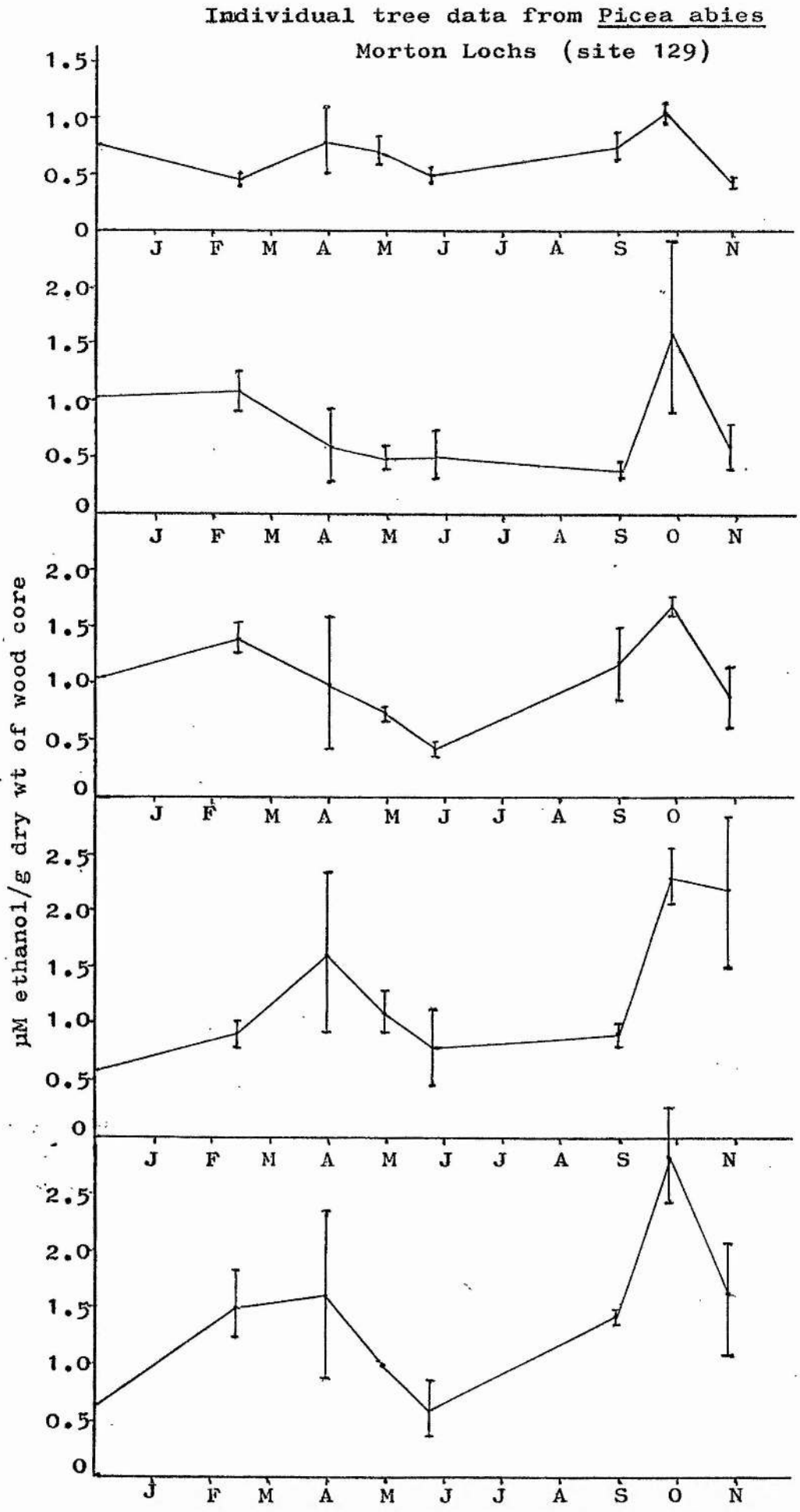

Individual tree data from Picea abies
Morton Lochs (site 129)
µM ethanol/g dry wt of wood core
J F M A M J J A S O N
Months from Nov. 1975 to Dec. 1976

Fig.II/7

Fig. II/8

Monthly levels of ethanol (μM ethanol/ g dry wt) detected in wood cores from the base of trunks of mature trees of Pinus sylvestris growing on a former dune slack (site 36) Tentsmuir, Fife.

Each graph represents the levels of ethanol detected in each of five individual trees. Each point is based on the mean of two estimates found using two core samples from each tree.

Vertical bars show maximum and minimum values recorded.

Prior to May the trees at this site were sampled randomly, therefore data for individual (marked) trees is only available from May 1976 onwards.

Individual tree core data from

Pinus sylvestris , former dune-slack ,

(site 36)

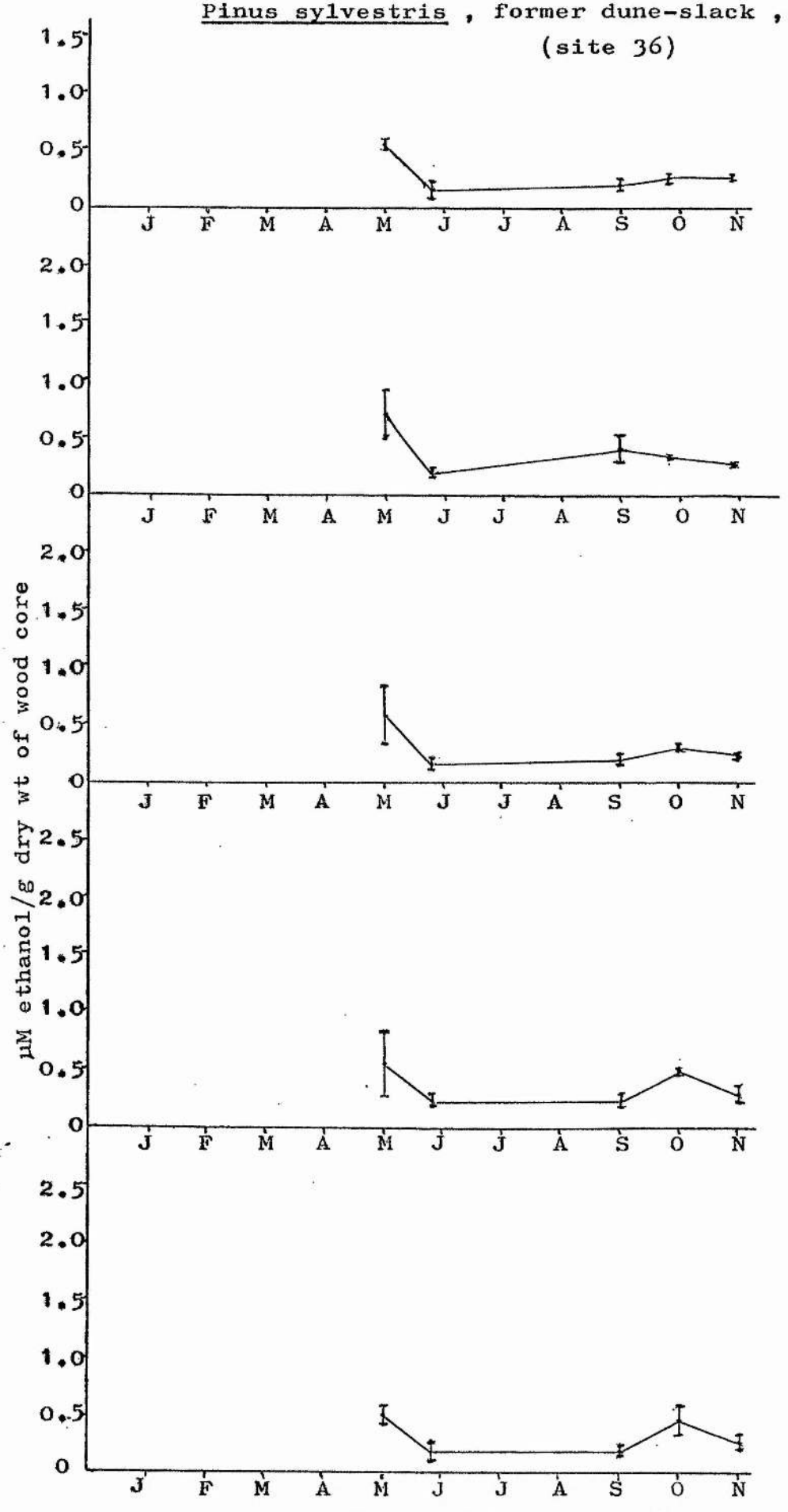


Fig. II/8

Fig. II/9

Monthly levels of ethanol (μM ethanol/g dry wt) detected in wood cores from the base of trunks of mature trees of Pinus sylvestris growing on an ancient sand dune (site 48) at Kinshaldy, Tentsmuir, Fife.

Each graph represents the levels of ethanol detected in each of five individual trees. Each point is based on the mean of two estimates, found using two core samples from each tree.

Vertical bars show maximum and minimum values recorded.

Prior to May, the trees at this site were sampled randomly, therefore data for individual (marked) trees is only available from May 1976 onwards.

Individual tree core data from

Pinus sylvestris, ancient sand dune,

(site 48)

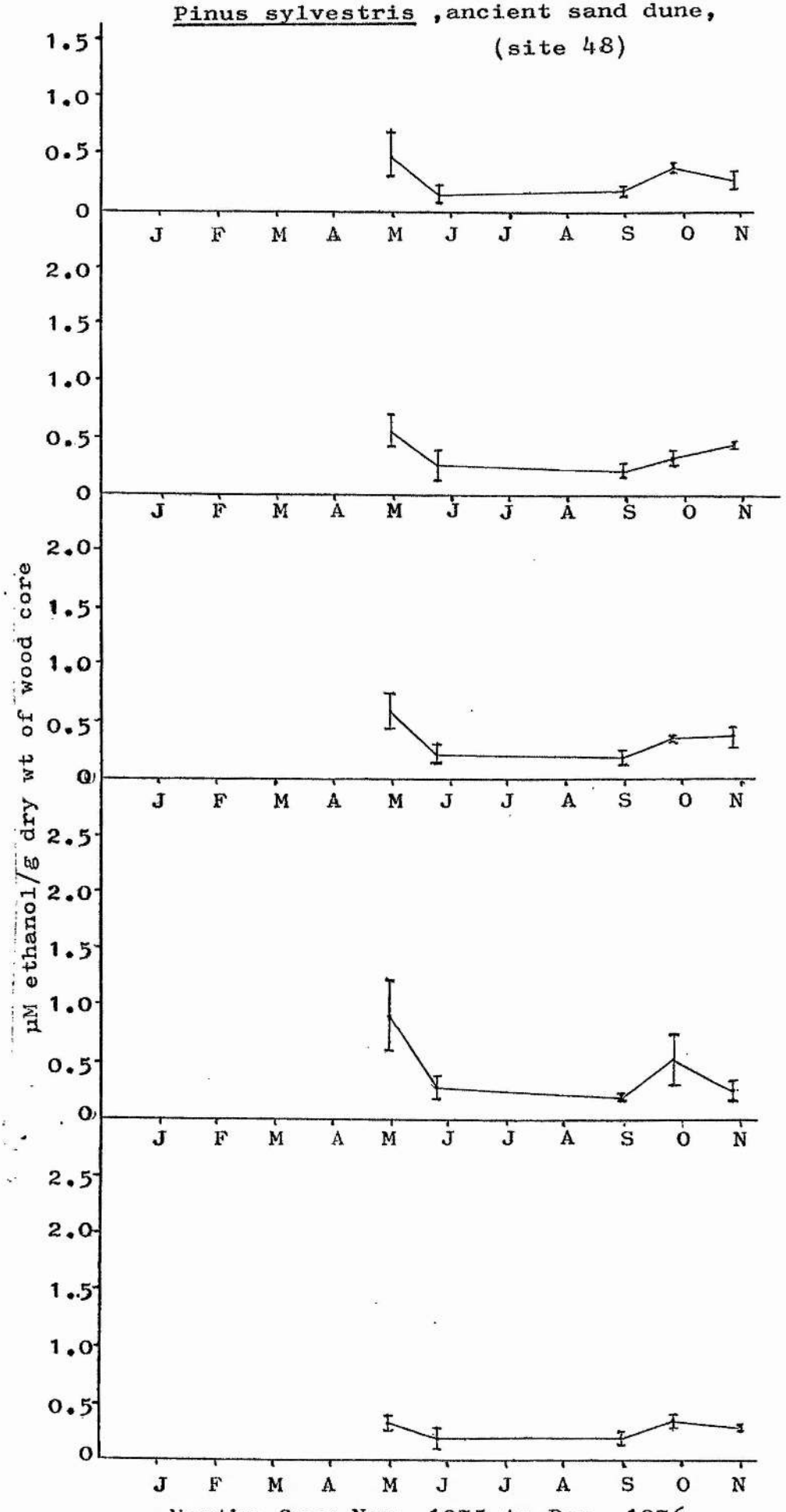


Fig.II/9

Fig. II/10

Monthly levels of malate (μM malate/ g dry wt of wood core), detected in wood cores from the base of trunks of mature trees of Picea abies and Pinus sylvestris growing at Tentsmuir, Fife.

- ● Picea abies growing in wet site with prolonged winter flooding, Morton Lochs (site 129)
- ▲ Pinus sylvestris growing on a former dune slack (site 36)
- △ Pinus sylvestris growing on an ancient sand dune, Kinshaldy, (site 48)

Error bars are based on ten separate estimates using two samples from each of five trees.

Malate detected in wood cores from trees of Picea abies and Pinus sylvestris, collected throughout the year at Tentsmuir, Fife

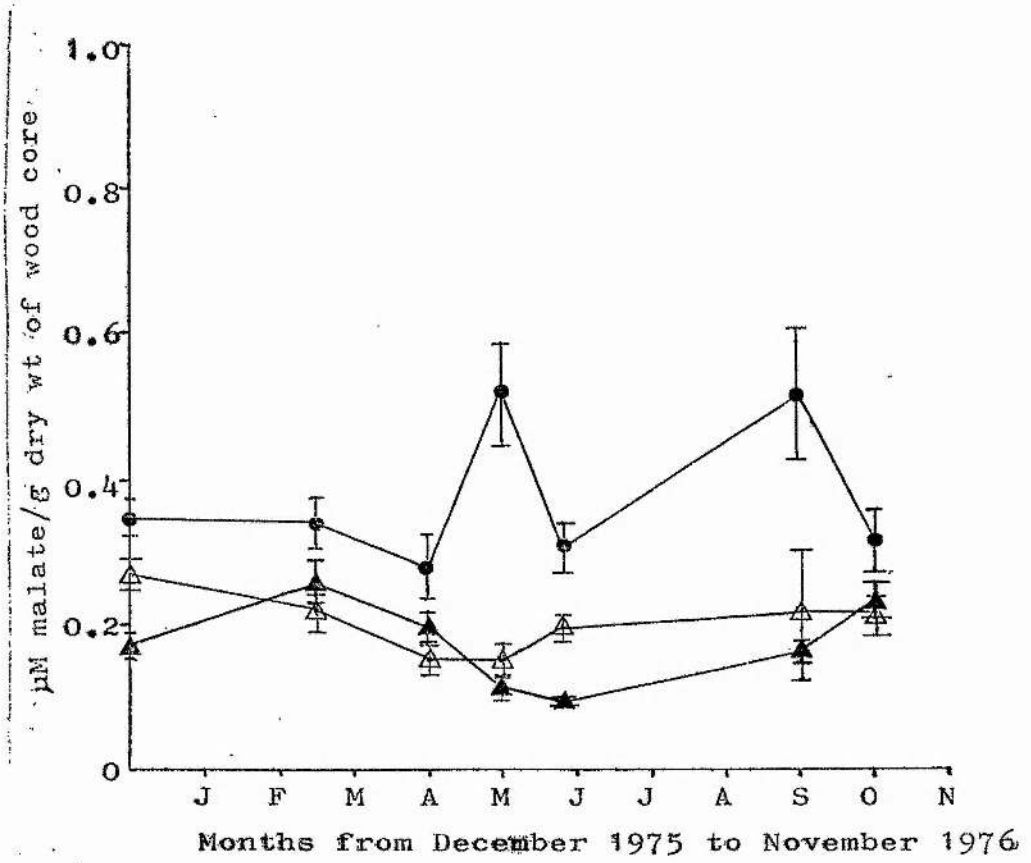


Fig. II/10

2. RESULTS

An analysis of the ethanol content of the trunk cores collected "dry", and root samples from the same trees, showed that there is a good degree of correlation ($P = 0.01$), (fig. II/3). There is also a significant correlation between ethanol content of cores collected "dry" or in perchloric acid ($P = 0.01$), (fig. II/4), although values are naturally higher in the latter. However, since it is the comparative results which are of main interest, the simplest method of field collection was used.

The results of the analysis of wood cores collected from the three sites over a year, are presented in fig. II/5. The first feature noticeable from these graphs is that, in both species, ethanol content of the wood cores is highest throughout the winter and in early spring. These findings support the conclusion that the ethanol estimated in the xylem sap sampled at the base of the tree, reflects the degree of anoxia to which the roots are subjected. However, the sharp rises in ethanol which occur in April and early May for the trees growing on the better drained sites, coincide with the period of resumption of metabolic activity after the winter, and so, in this case, the rise in ethanol may also be an indication of increase in oxygen demand. It is clear from the graphs, that, as expected, the highest ethanol contents are found for those trees (Picea abies) growing on the wettest site, although it is also obvious that readily detectable levels of ethanol are found in cores of all species throughout the year.

Fig. II/6 shows that the fluctuation in depth of water table in a dune slack at Tentsmuir in 1964 can be correlated, not with rainfall, but rather, with the temperature. This is because, in sand dune areas, stream outlets are few, and evapo-transpiration, which is largely temperature dependant, is the controlling factor determining the rate of water removal. Thus, as can be seen in fig. II/6, the period of maximum height of the water table, is found in the

coldest months of the year, and the maximum amount of flooding of root systems will always be expected at this season. Comparison of figs. II/5 and II/6 show that maximum and minimum levels of ethanol in cores of all species, coincide with the predicted times of high and low water table respectively. Figs. II/7/8/9 indicate ethanol concentrations in individual trees.

Fig. II/10 shows the corresponding amount of malate found in the same wood cores over the year. The amounts of this metabolite in the three sets of trees correspond in magnitude with the amounts of ethanol found in the respective sets. Although the amount of malate found throughout the year does vary to some extent, the seasonal correlations and the amount of fluctuation is not so great as that observed for ethanol.

3. DISCUSSION

As long ago as 1899, Devaux showed that anoxic conditions prevail within trees, even in normal environmental conditions, simply by virtue of their structure. This was also recognised by Hook et al (Hook, Brown and Wetmore 1972). More recently, Carodus and Triffett (1975) carried out an analysis of respiratory gases in woody stems by mass spectrometry, and their results confirm those of Devaux. The prevalence of anoxic conditions and the detection of ethanol by Devaux in his wood distillates, are both re-affirmed by the findings presented in this chapter. Readily detectable quantities of ethanol were found in wood core samples from both species throughout the year - not only when the water table levels were high. This indicates that at all times, at least part of the root systems of these trees, are in a state of anoxia. There has been much controversy over this assertion, since some workers, notably Armstrong (1967) have contended that survival of anoxic soil conditions is not made possible by the ability of the plant to cope with anoxia, but by the ability to avoid it. This is achieved by the aeration of the roots with oxygen from the leaves, or lenticels. Aeration is undoubtedly very important in many species, especially

herbaceous wetland plants (Krasovskii and Chashchukhim 1974; van Raalte 1941. Oxygen diffusion has, however, been reported in some woody species too (Armstrong 1968; Chirkova 1968; Hook et al 1971; Huikari 1954; Leyton and Rousseau 1958). Willow species are reported to be able to survive almost complete oxygen exclusion (Livingstone and Free 1917) and to achieve this by transporting oxygen through the trunk to the roots (Armstrong 1968). This aeration is probably provided for by the marked development of lenticels at the base of the trunk. However, the distance over which this diffusion occurs is relatively short, and ceases when the lenticels at the base of the tree are covered with water to a depth of 3cm (Armstrong 1968).

Internal aeration has been reported in the two flood tolerant trees Nyssa aquatica and Fraxinus pennsylvanica (Dickson, Hosner and Hosley 1965; Hosner and Boyce 1962). Here aeration appears to operate through intercellular spaces in the cambium (Hook, Brown and Wetmore 1972). Gas filled intercellular spaces have been observed in the medullary rays of the two species studied in this chapter Picea abies and Pinus sylvestris (Back 1969; Ziegler 1964).

It is probable that diffusion aids the aeration of adventitious roots produced near the surface, and other shallow roots, but that it has no marked effect on the deeper mature roots. Coutts and Philipson (1978) report the survival of Pinus contorta roots about 1m below the water table, and concede that this may be explained by some modification of respiratory mechanism, since 1m is in excess of published distances of oxygen transport. Wooley (1965) has calculated that even for a corn root with 8% intercellular spaces, only 10cm of root could be totally supported by oxygen diffusing from higher in the plant. Vatapetian and Nuritidinov (1976) have shown that in Gossypium, shoot to root diffusion is only able to supply 8% of the oxygen required by the roots.

Although it is true that internal aeration is probably important in some species, it is worth remembering that oxygen has been found to be transported from the shoots to the roots of a number of herbaceous species, including onion,

pea, lettuce, leeks, beetroot, buckwhat, turnip, barley, rye grass, carrot, cabbage (Greenwood 1967) and corn (Jensen, Letey and Stolzy 1964), yet none of these species are tolerant of flooded conditions.

Despite the various anatomical adaptions which do occur in some cases to aid aeration of the root, it is apparent that, in the species studied in this chapter, anoxic conditions still prevailed, since ethanol is produced. As mentioned in the previous chapter, the production of ethanol occurs only in anaerobic conditions. It has been suggested (Huck and Erickson unpublished), that much of the ethanol that was present in the xylem sap of tomato, cotton and soybean plants, was in fact microbial in origin. This may again be a feature which must be taken into consideration when interpreting ethanol levels in trees; some of the ethanol recovered may be produced by mycorrhizal fungi although Coutts and Armstrong (1976) state that mycorrhizal fungi cannot grow anaerobically and Coutts and Philipson (1978) found that mycorrhizae on the roots of Pinus contorta and Picea sitchensis were restricted to the aerated soil horizon. Kramer (1951a) has shown that mycorrhizal and non-mycorrhizal roots of loblolly pine react differently to respiration inhibitors. However, mycorrhizal fungi are always present "in the field" and from the point of view of using ethanol as a measure of soil aeration and plant damage, it is largely irrelevant where the ethanol originates, since its presence above a certain level indicates bad soil aeration, and if its concentration is high enough, it will cause damage, no matter what its origin.

The question arises, can ethanol levels really be used as a reliable measure of indicating plant and soil conditions in relation to water logging? Clearly the results shown in this chapter indicate that the method is a promising one, although much further work is obviously needed. The increase in ethanol levels which occurs during periods of high metabolic activity and oxygen demand in early spring, detract from its suitability for use as a measure of soil water

status. However, this is not the information which is really required (and it can, in any case, be measured more accurately by direct methods). The information which is required is the reaction of a certain tree to the combined environmental and Physiological conditions at the time, with respect to root respiration. It seems probable that a measurement of this - "how the tree is doing", could be found by monitoring ethand levels.

The present survey shows that there is good agreement between the levels of ethanol found in different individual trees of the same species on the same site (figs. II/7/8/9), and fig. II/5 shows that there is also good agreement in order of magnitude for the same species in two different sites. Obviously this brief survey would need to be extended to cover a longer time period, more sites, more species, and monitoring of the environmental conditions. However, in relation to extending the time period, further samples collected at Morton Lochs in March 1977, produced an ethanol value (1.02 µM ethanol/g dry wt.) very similar to that recorded in March 1976 (1.12 µM ethanol/g dry wt.). This gives some indication that the annual pattern shown in fig. II/5 is reproducible (although of course subject to variations of temperature and rainfall).

Those results suggest that, with further study, it may become possible to predict the range of ethanol levels likely to be encountered during a normal year, for a particular species, on a particular site. Extending predictions to a wider range of trees entails the difficulties of taking the various physical characteristics of the site into account. The density of the stands and the size of the root systems are also likely to have a bearing on the results. More ethanol may be detected in the base of the trunk of a tree with a large rooting system, than that detected in the base of the trunk of another tree in the same area, but with a smaller rooting system. This is because the ethanol measured represents the pool from a larger number of roots, although individual roots in both trees may be producing similar amounts of ethanol. What levels then are damaging to the trees concerned? This would initially have to be determined by relating the

amounts of ethanol measured in trees, to their subsequent history. Thus levels recorded from trees which recover from waterlogging could be compared to levels from trees which die following a period of waterlogging. Ethanol levels may perhaps also be calibrated to some extent by comparing them to the old measure of "force required to pull the tree over". In this way, it should be possible to determine the levels at which ethanol becomes potentially toxic to certain species, and also the tolerance of particular species to waterlogged conditions and/or ethanol levels. It may be that certain species may still be able to recover following periods of high ethanol production, which would kill other species (Chirkova 1975). Also according to the work of Crawford (1966) it is likely that there will be some flood tolerant species that will undergo a far smaller percentage increase in ethanol production with the onset of waterlogged conditions, than will be observed in other species which are not tolerant of flooding. This aspect is investigated more fully in the laboratory investigations discussed in the following chapter.

CHAPTER III

ROOT ETHANOL LEVELS AND FLOODING TOLERANCE

1. INTRODUCTION

The results of the previous chapter have shown that there may indeed be some relationship between degree of anoxia and ethanol levels in the roots of Pinus sylvestris and Picea abies. In this chapter laboratory experiments are described which were carried out both to quantify more fully the relationship between ethanol levels in flooded and unflooded conditions, and also to investigate the relationship between ethanol production in anoxic conditions and sensitivity to flooding. The investigations were carried out using two year old seedlings of Picea sitchensis and Pinus contorta set up in water culture and in pots in the glasshouse. Previous work (Boggie 1974; Vester 1972); (Coutts and Armstrong 1976; Coutts & Philipson 1978), has shown that Picea sitchensis is relatively flood intolerant, and that Pinus contorta is relatively flood tolerant (see plate 1).

Ethanol content of roots from flooded and unflooded pots and aerated and nonaerated water culture were determined enzymatically. (Experimental Series D).

2. RESULTS

As with the field experiments, ethanol could always be detected in the roots of the water cultured and pot grown seedlings. This shows once again that both species are suffering from hypoxia in aerated as well as unaerated conditions. Ethanol levels were never-the-less, much higher in unaerated conditions, being double the aerobic levels 12 hours after the cessation of aeration of the water culture in both species. However, beyond 12 hours of anaerobiosis, the concentration of ethanol was far higher in the flood intolerant Picea sitchensis (fig. III/1), a maximum of over 6.0 µM/ethanol/g.fr.wt., compared to below 1.0 µM ethanol/g.fr.wt., as found in Pinus contorta.

PLATE 1

Effect of prolonged flooding by pot submersion of trees grown under glass. The unflooded control trees are on the left and the flooded on the right.

No. 1 *Picea sitchensis* flooded for three months

No. 2 *Pinus contorta* flooded for four months

PLATE I

Fig. III/1

Accumulation of ethanol in root of Pinus contorta (▲) and Picea sitchensis (●), grown in water culture after cessation of aeration.

The vertical bars represent the maximum and minimum values obtained with three different trees.

Accumulation of ethanol in roots of Pinus contorta and Picea sitchensis in water culture, after cessation of aeration

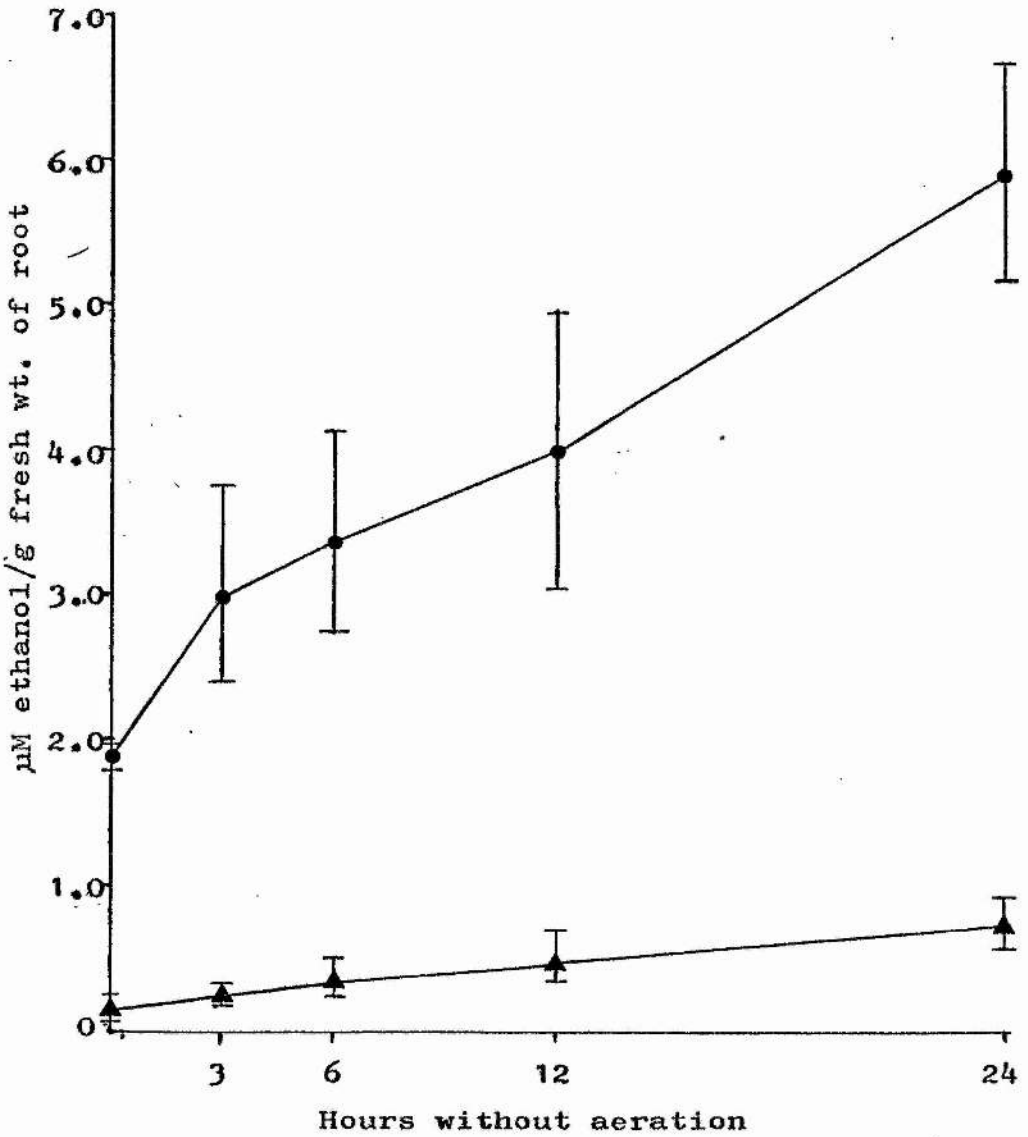


Fig.III/1

Fig. III/2 .

Ethanol content of roots of Pinus contorta and Picea sitchensis flooded in pots for 24 hours. The vertical bars indicate the maximum and minimum values obtained for four different trees.

Hatched columns, ethanol content of roots in unflooded pots; open columns, ethanol content of roots from flooded pots.

Ethanol content of roots of Pinus contorta and Picea sitchensis in flooded or unflooded pots

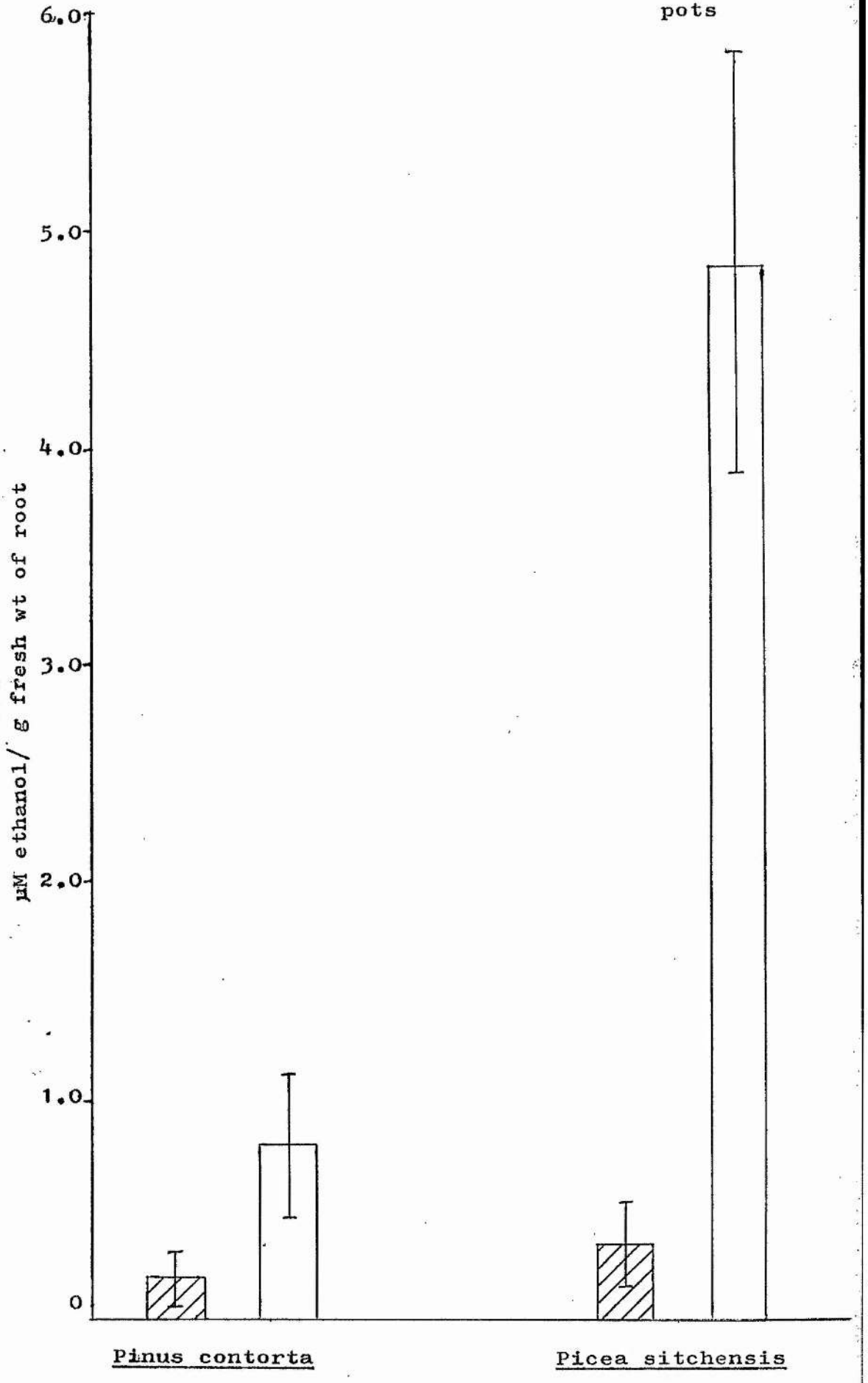


Fig III/2

Picea sitchensis also showed abnormally high values for ethanol even in aerated water culture (1.9 µM ethanol/g. fr.wt., see 0 h fig. III/1). It was to obviate this possible premature induction of anaerobiosis that the trees were flooded while still potted in the 1:1 mixture of peat and sand. Under these conditions, Picea sitchensis exhibited a lower ethanol concentration in the roots before flooding than it did in aerated water culture (fig. III/2). This "aerobic" level of ethanol found by this method was comparable to that found for Pinus contorta in the same conditions. The results for Pinus contorta and flooded Picea sitchensis are comparable to those found in the water culture experiment. Similar agreement between ethanol levels in roots of both pot grown and water cultured plants was previously found by Crawford (1967), when investigating a range of Senecio species.

When the two species are compared after 24 hours of flooding, (fig. III/2) it can be seen that the flood intolerant Picea sitchensis showed a twelve fold increase in root ethanol after 24 hours flooding, compared with only a three fold increase in root ethanol in Pinus contorta.

A further experiment in Experimental Series D, using Picea sitchensis in open but unaerated water culture, gave an ethanol level of 7.14 uM ethanol/g.fr.wt. after 72 hours. It appears therefore that the ethanol level continues to rise.

3. DISCUSSION

It can be seen from these results that these two species, one sensitive to flooding and one not, react differently when their roots are subjected to anaerobic conditions. The roots of the flood intolerant Picea sitchensis show a marked increase in glycolysis as evidenced by the greatly increased ethanol production. The flood tolerant Pinus contorta showed very little increase in glycolytic activity. Crawford (1967) has linked the increase in glycolytic activity, such as occurs in Picea sitchensis, with the activation of alcohol dehydrogenase (ADH). He suggests that plants in which this activation occurs are

excluded from growing in wet areas because of the accumulation of toxic quantities of ethanol. McManmon and Crawford (1971) showed again that in a number of species, sensitivity to flooding could be matched by a rapid marked induction of ADH activity in flooded conditions. Other workers have shown that, in a number of cases involving varieties of the same species, degree of flooding tolerance can in fact be traced to ADH isoenzyme differences, (Marshall, Broue and Oram 1974; Marshall, Broue and Pryor 1975; Francis Devitt and Steele 1974). The work on Lupinus angustifolium (Marshall, Broue and Oram 1974) showed that plants which contain the ADH_1 (fast) allele, have readily inducible ADH activity, and are always sensitive to flooding. The other allele, ADH_2 (slow), is found in plants with less ADH activity and greater ability to withstand flooding.

Thus sensitivity to flooding can often be linked with increased ADH activity and increased ethanol production when flooding occurs. However, a number of flood tolerant plants have also shown these increases, but these reports come only from work on adventitious unsuberised root systems of tropical plants. John and Greenaway (1976) found an increase in ADH activity in rice roots when flooded, and Hook, Brown and Kormanik (1971), showed that in seedling roots of Nyassa aquatica, flooding caused an increase in ethanol production, combined with increased root porosity and reduced suberisation. Under these conditions, where thin root tissue is in contact with the external aquatic medium, ethanol will diffuse readily from the flooded tissues, and may not cause the harmful effects that otherwise are always associated with its accumulation.

As previously discussed in Chapter I, these harmful effects of ethanol, may be related to its lipophillic properties. This mode of action would explain the accelerating effect of anoxia on the rate of glycolsis in flood sensitive roots (Crawford 1966). This is an autocatalitic effect, produced by the action

of ethanol in attacking membranes and inhibiting aerobic respiration. Any reduction in ATP concentration will be likely to initiate the Pasteur Effect (Krebs 1972) in flood intolerant tissues, thus causing further increases in ethanol, and greater disruption of lipid membranes. In this way relatively short periods of anoxia can initiate the rapid ethanol poisoning of roots.

Despite the evidence for a difference in anaerobic root metabolism between Pinus contorta and Picea sitchensis, Coutts and Armstrong (1976) and Coutts and Philipson (1978 I and II) have also provided evidence to show that there may be a difference in the ability of these two species to transport oxygen from their shoots to their roots, thereby avoiding anoxia to some extent. Coutts and Armstrong (1976) describe broad zones of air filled tracheids sometimes present in the flood tolerant Pinus contorta, but absent from the flood intolerant Picea sitchensis. However, Armstrong and Read (1972) previously showed that the oxygen flux from the roots of both of these species, decreases rapidly when oxygen is prevented from reaching the shoots, while Boggie (1974) could detect no oxygen diffusion from the roots of Pinus contorta. There is no evidence from the work described in this chapter that Pinus contorta delays the onset of anaerobiosis in unaerated water culture by oxygen diffusion from shoot to root. The equally rapid depletion of oxygen in the culture vessels (fig. I/1) of both species, together with the simultaneous rise in ethanol production, indicates that as soon as aeration ceases, anoxia increases. However, the possible physical presence of oxygen in portions of the roots of Pinus contorta cannot be excluded.

Coutts and Armstrong (1976) have suggested that the products of anoxia may induce the formation of embolisms in the xylem, leading to more developed gas ways such as those they found in Pinus contorta. However, it is interesting to speculate why the products of anoxia should produce these embolisms in Pinus contorta, but not in Picea sitchensis, especially when the evidence

reported in this chapter suggests that the products of anoxia are in greater abundance in Picea sitchensis. It may too be of importance that the lacunae in Pinus contorta do eventually close anyway due to the growth of the secondary xylem. Nevertheless, Coutts and Philipson (1978 II) did observe that, though the spaces in the pericycle of Pinus contorta, were present in roots of plants from both waterlogged and well drained soil, these spaces were much larger, and longitudinally continuous in those plants from waterlogged soil. Coutts and Philipson (1978 II) also found that roots of Pinus contorta could penetrate waterlogged peat to a maximum depth of 167 mm, though growth rate decreased with increasing depth. However, if roots which had penetrated the water table only 20 mm, were then flooded to a depth of 100 mm for 10 days, extension growth stopped, although the roots survived and extension growth continued again after drainage. Roots which were flooded to 100 mm without having previously grown into the water table, were severely affected; 96% of the root tips being dead after 10 days waterlogging.

The results of the work conducted in this chapter, and that of other workers, suggest that there is some form of gradual adaption to waterlogging - probably the provision of air spaces - which will provide not only for the survival of the roots, but also their continued though limited, growth, down into the waterlogged soil. Survival of sudden flooding cannot depend on the formation of these air spaces, but their prior existance, and the resultant higher residual of oxygen remaining in the root, may be required to allow time for survival measures to come into action. It is likely that these will entail some form of metabolic adaption. Sudden flooding of totally un-preconditioned roots apparently kills them so rapidly, that there is not time for even a metabolic modification to come into force.

The decline of root extension with depth is consistant with dependance upon oxygen transport because oxygen is lost to respiration and by leakage during

passage down the root (Coutts and Philipson 1978 II). However, continued survival of Pinus contorta about 1 m below the water table (Coutts and Philipson 1978 II) which is in excess of published distances of oxygen transport, is consistant with some form of metabolic adaption.

Clearly aeration of the roots from the shoots plays an important part in helping to preserve the viability of plant roots in flooded conditions. However, this mechanism does not always operate in isolation, but may be accompanied by the ability to control metabolic rate, and production of ethanol. This would appear to be the case for Pinus contorta since there is good evidence for transport of oxygen from shoot to root (Coutts and Armstrong 1976; Coutts and Philipson 1978 I and II), but also, as detailed in this chapter, good evidence for metabolic control, which may cover many aspects, but included the ability to control the rate of glycolysis in anaerobic conditions, and to prevent the accumulation of toxic levels of ethanol.

CHAPTER IV

ETHANOL TOXICITY: THE EFFECT OF EXTERNAL ETHANOL

1 INTRODUCTION

It appeared from the last experiment that a major reason for the difference in flood-tolerance between the flood intolerant Picea sitchensis and the flood tolerant Pinus contorta, could lie in their unequal accumulation of ethanol in anaerobic conditions.

Although some workers (Lundegardh 1948) consider ethanol as a harmless by-product of anaerobic respiration, others (Fulton and Erickson 1964; Wooley 1965) have held that it may be high levels of ethanol which have proved lethally toxic to those plants which die as a result of exposure to anaerobic root conditions. Although this has been a fairly widely held presumption, there is little hard evidence, apart from that concerning micro-organisms, that this is actually so. In an attempt to elucidate this situation, experiments were carried out to try and determine the effects of different concentrations of exogenous ethanol on the root.

2 ROOT LEAKAGE UNDER THE INFLUENCE OF ANOXIA AND EXTERNAL ETHANOL

The toxic action of ethanol may be via its action as a lipid solvent, fluidising membranes, and thereby leading to complete disruption of the metabolism. As discussed in Chapter I, the increase in leakage of substances from roots under anaerobic conditions which has been observed (Christiansen et al 1970; Grineva 1962; Hiatt and Lowe 1967; Lundegardh 1948; Marschner et al 1966; Simon 1974) may be due to a combination of several reasons:-

a) Lack of energy required to maintain membrane integrity

b) Solvent action of ethanol

c) Promotion of active excretion due to reduction in water absorption

d) Following increasing leakage and subsequent increase in acidity of the surrounding medium, there may be an additional effect of H ions on membrane disruption and ion exchange.

If the solvent action of ethanol does play a major role in the breakdown of cellular organisation and ultimate death of roots in anaerobic conditions, then increasing leakage from roots in higher ethanol concentrations, would be expected to be symptomatic of the effect.

2. A. Results

The results of this experiment (see Experimental Series C), express leakage from the roots of Pinus contorta and Picea sitchensis, into the external medium, in terms of -

a) Potassium ion concentration

b) Conductivity

c) pH

These measurements all refer to the external root medium. Since root volumes varied between both species and individuals, it was considered more accurate to record potassium concentration and conductivity as ratios of these root volumes.

POTASSIUM: The leakage of potassium into the medium surrounding the roots of Pinus contorta seedlings, shows a general increase corresponding to increase in ethanol concentration. The differential effects of the varying treatments become more marked with time (fig. IV/1). The potassium concentration reaches 1.45 mg/1/cc root (or 24.1 mg/1), in the medium containing 10^{-1}M ethanol, compared to only 0.58 mg/1/cc root (or 13.6 mg/1), in pure (but also unaerated) water culture. Aerobic values are lower still. The histogram (fig. IV/2) shows that, while in each case there is a high initial level of potassium leakage, probably from the free space, a high level of leakage is only maintained at the higher ethanol concentrations.

The effect of external ethanol on leakage of potassium from roots of Picea sitchensis is far from clear (fig. IV/3). This is in accord with the results from the short term experiment (Exp. Series A) discussed in Chapter I, where the

effects of aerobic and anaerobic conditions on potassium leakage were compared. Here too, though the effect of the conditions were clearly marked in Pinus contorta there was no clear differentiation between treatments in Picea sitchensis. In Experimental Series A, it appears, that there is greater leakage from Picea sitchensis roots in aerobic rather than in anaerobic conditions. As pointed out in Chapter I, this is also true in Experimental Series C, further results of which are being discussed in this chapter. The results shown in fig. IV/3 indicate that, for Picea sitchensis the root medium containing the highest concentration of potassium after 14 days in Experimental Series C, is the aerated distilled water. The value here is 0.76 mg/l/cc root (or 9.25 mg/l). This is similar to the potassium concentration found in aerobic conditions for Pinus contorta, 0.36 mg/l/cc root (or 8.35 mg/l), but in this case they are the lowest rather than the highest recorded. The histogram (fig. IV/4), shows that the 10^{-1} M ethanol medium had the highest initial level of potassium loss for Picea sitchensis but that there was little further increase.

CONDUCTIVITY: For Pinus contorta conductivity of the external medium shows the same general trends as are displayed for potassium concentration; an increase with both time and ethanol concentration (fig. IV/5). There is a high initial increase in conductivity in aerobic conditions, but this level then remains fairly steady. The effects of external ethanol are most clearly defined at the higher ethanol concentrations, and again, it is only at these concentrations that there is a continued high level of increase in conductivity (fig. IV/6).

For Picea sitchensis there is a low level of conductivity in all treatments compared to those found for Pinus contorta (fig. IV/7). This corresponds with the levels of potassium found in this experiment, and, as is found for potassium, the highest conductivity is that of the aerated distilled water, 7.34 µmho/cc root (or 89.25 µmho), compared to 6.32 µmho/cc root (or 140.0 µmho), for the same

treatment in Pinus contorta. This finding is in agreement with those from Experimental Series A, (Chapter I), where again, the highest conductivity for Picea sitchensis is that of aerated distilled water, whereas that for Pinus contorta, is found in the water where aeration had been discontinued the longest. The conductivity of the 10^{-1}M ethanol medium is in fact the lowest in Picea sitchensis, if expressed as 37.05 µmho, though the second lowest if expressed as 4.94 umho/cc root, compared to the highest in Pinus contorta 17.85 µmho/cc root (or 311 µmho). The histograms (fig. IV/8) show (as they did for potassium) that there is a high initial increase in conductivity in the 10^{-1}M ethanol solution for Picea sitchensis, but that this level of increase is not maintained, and is later surpassed in other treatments.

pH: pH levels were monitored in a number of experiments, and were always found to increase in aerated water culture, and decrease in anaerobic water culture, in both species. However, the extent of increase and decrease does seem to depend on the species, and, as shown here, the decrease in anaerobic conditions is also affected by the presence of ethanol around the root. (fig. IV/9 & IV/10).

Pinus contorta shows a clear correlation between ethanol treatment and pH of the medium, but Picea sitchensis, again fails to show any well marked effects of different ethanol concentrations. The overall decrease in pH in unaerated conditions for Picea sitchensis, both in distilled water and in ethanol, is less than that observed in Pinus contorta, and the range covered by the pH of Picea sitchensis is narrower than that for Pinus contorta. The minimum value for the unaerated Picea sitchensis is pH 5.54 (in the 10^{-2}M ethanol treatment), after 14 days, and the maximum value (found in the 10^{-4} M ethanol treatment) is pH 5.8. In the case of Pinus contorta the minimum value in unaerated culture is that for the 10^{-1}M ethanol treatment, and is pH 4.38, whilst the maximum (for both the unaerated distilled water and the 10^{-4}M ethanol treatments) is pH 5.5. (similar to the minimum value for Picea sitchensis).

Fig. IV/1

The potassium content (mg l^{-1}/cc root) around roots of Pinus contorta seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

- - - aerobic
——— anaerobic

} no added ethanol

- -△- 10^{-4}M ethanol
—▲— 10^{-3}M ethanol
—△— 10^{-2}M ethanol
- -▲- 10^{-1}M ethanol

} anaerobic

Each point represents the mean value for two culture vessels.

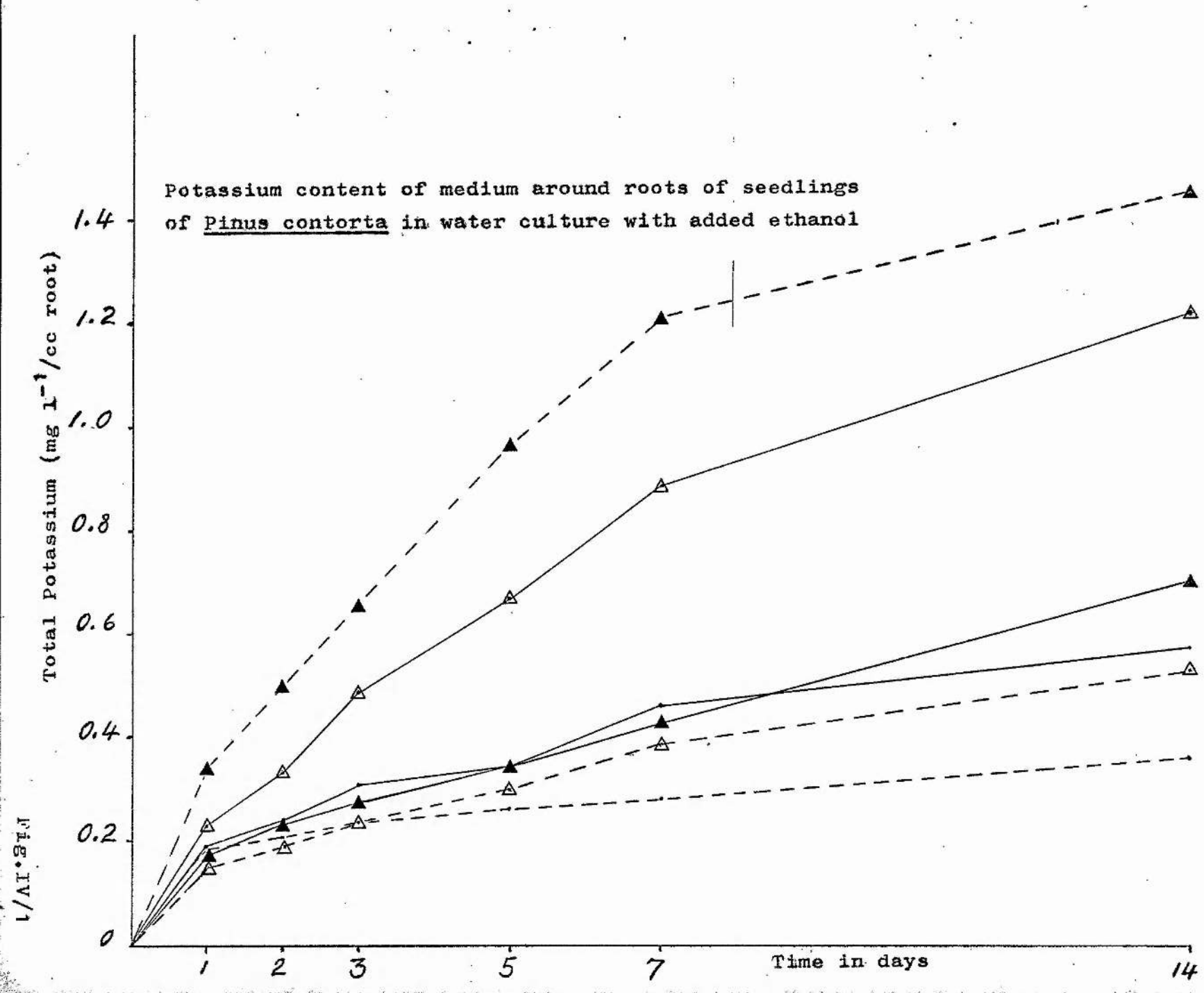

Potassium content of medium around roots of seedlings
of Pinus contorta in water culture with added ethanol
Total Potassium (mg l⁻¹/cc root)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
1
2
3
5
7
14
Time in days

Fig. IV/1

Fig. IV/2

Histograms of potassium content (mg l^{-1}/cc root/day) of medium around roots of Pinus contorta seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels, as indicated.

Experimental Series C.

Each column represents the mean value for two culture vessels.

aerobic, anaerobic } no added ethanol

10^{-4}M ethanol, 10^{-3}M ethanol, 10^{-2}M ethanol, 10^{-1}M ethanol } anaerobic

Potassium content of medium around roots of seedlings of Pinus contorta in water culture with added ethanol

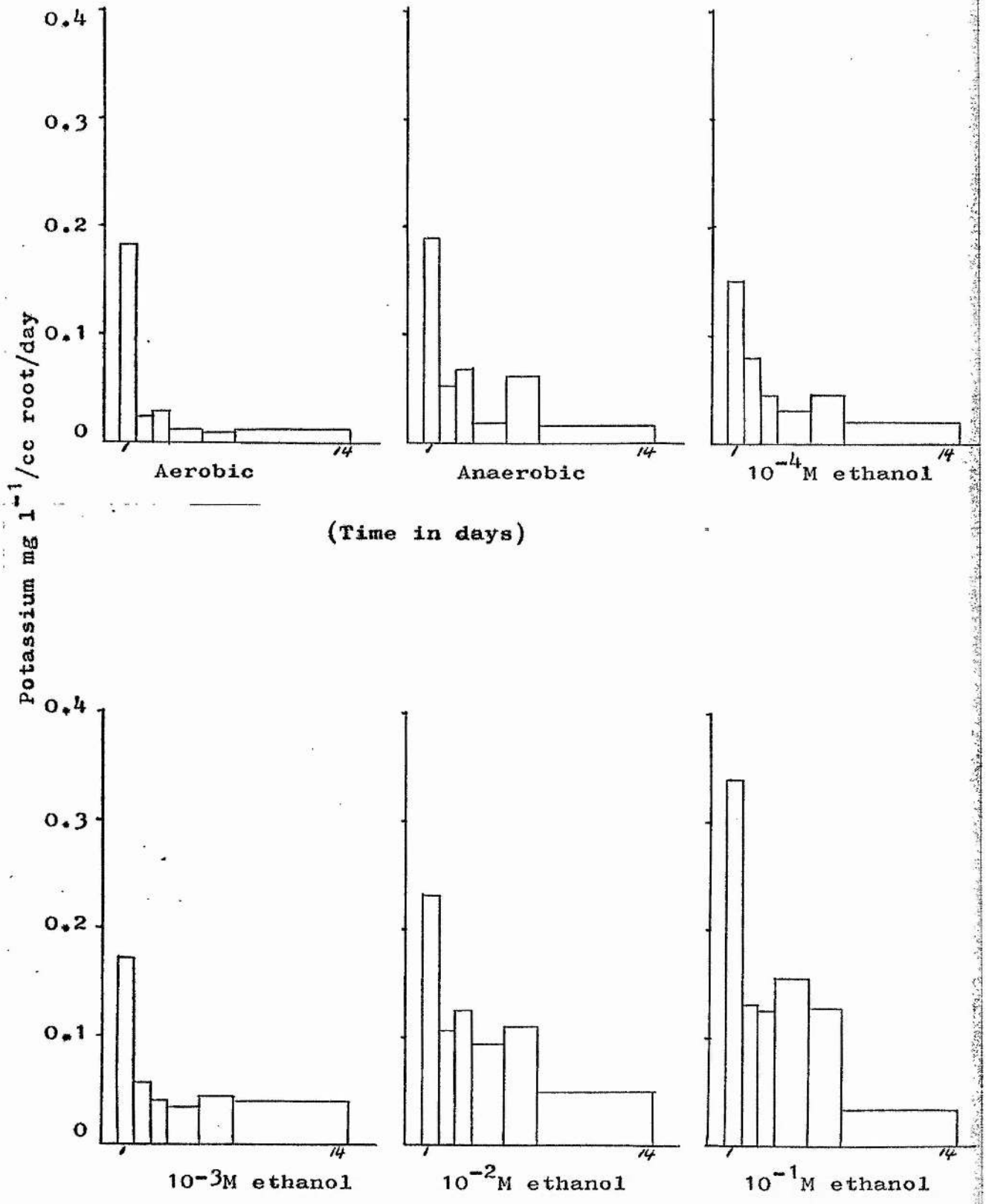


Fig.IV/2

Fig. IV/3

The potassium content (mg l^{-1}/cc root) around roots of Picea sitchensis seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

- - - aerobic
——— anaerobic
} no added ethanol

- ⊖ - 10^{-4}M ethanol
—●— 10^{-3}M ethanol
—⊖— 10^{-2}M ethanol
- ● - 10^{-1}M ethanol
} anaerobic

Each point represents the mean value for two culture vessels.

Potassium content of medium around roots of seedlings of
Picea sitchensis in water culture with added ethanol

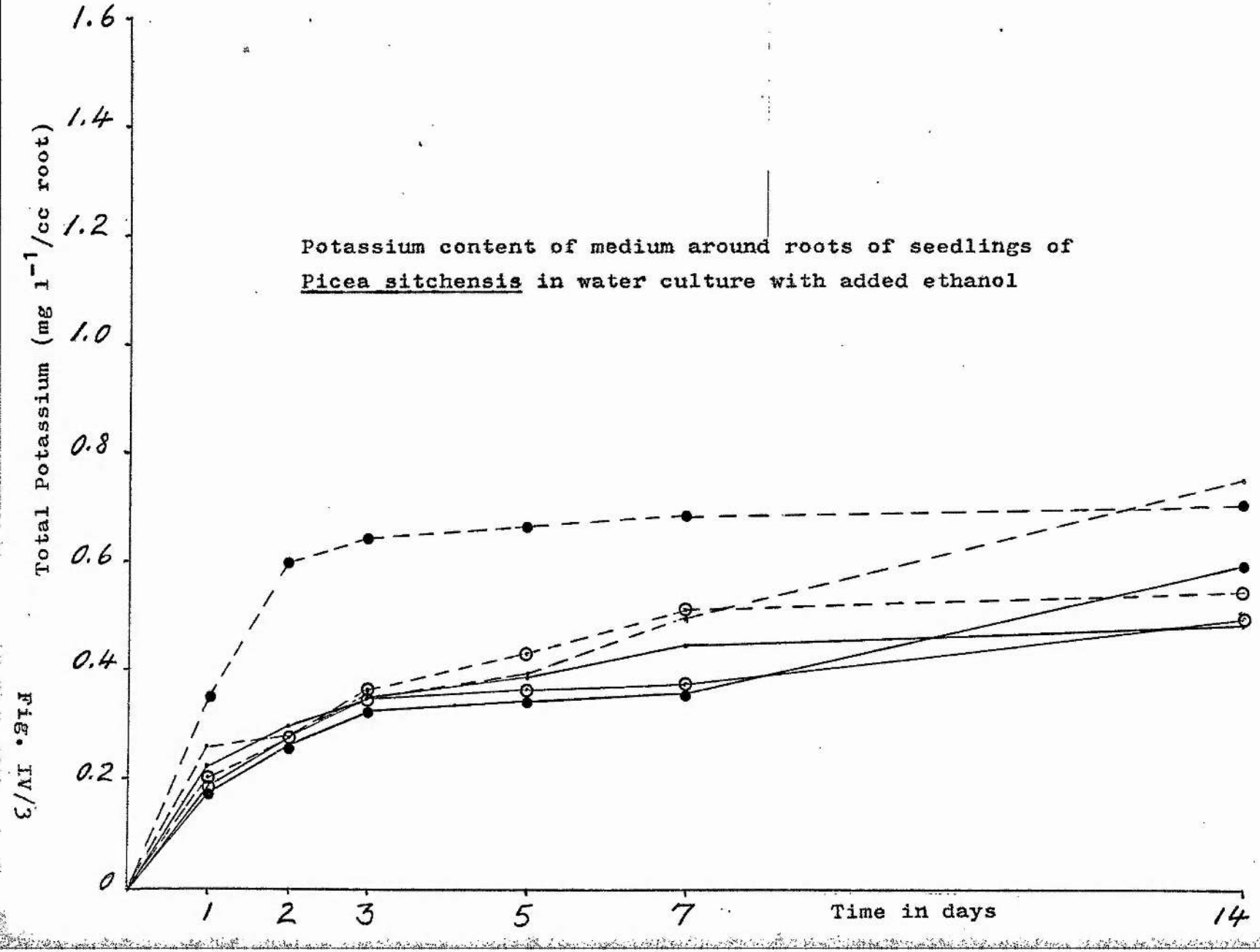


Fig. IV/3

Fig. IV/4

Histograms of potassium content (mg l^{-1}/cc root/day) of medium around roots of Picea sitchensis seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of ethanol in the sealed vessels, as indicated. Experimental Series C.

Each column represents the mean value for two culture vessels.

aerobic } no added ethanol
anaerobic }

10^{-4} ethanol }
10^{-3} ethanol } anaerobic
10^{-2} ethanol }
10^{-1} ethanol }

Potassium content of medium around roots of seedlings of Picea sitchensis in water culture with added ethanol

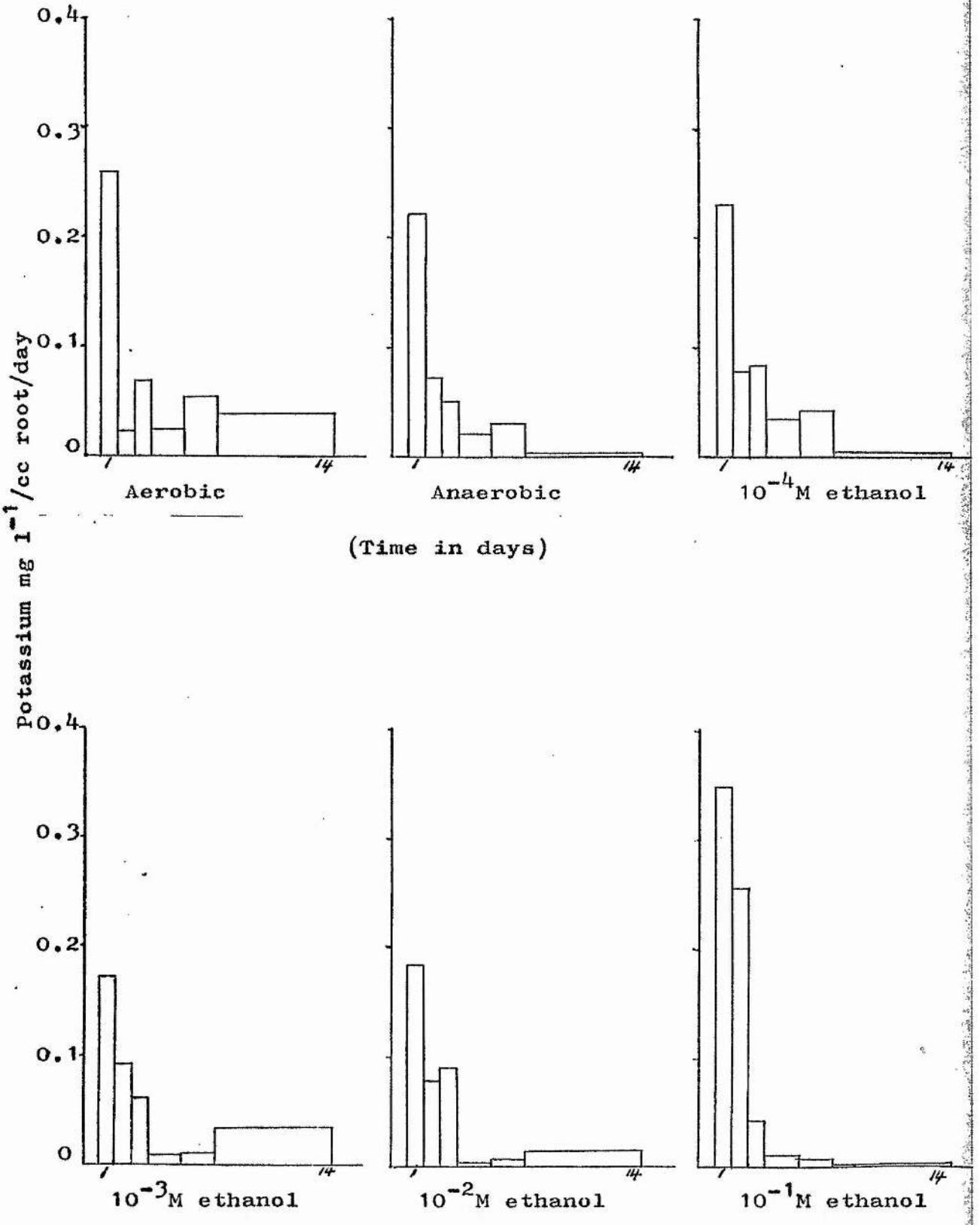


Fig.IV/4

Fig. IV/5

Conductivity (μmho/cc root) of medium around roots of Pinus contorta seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

- - -	aerobic	no added ethanol
———	anaerobic	
-△-	10^{-4}M ethanol	anaerobic
—▲—	10^{-3}M ethanol	
—△—	10^{-2}M ethanol	
-▲-	10^{-1}M ethanol	

Each point represents the mean value for two culture vessels.

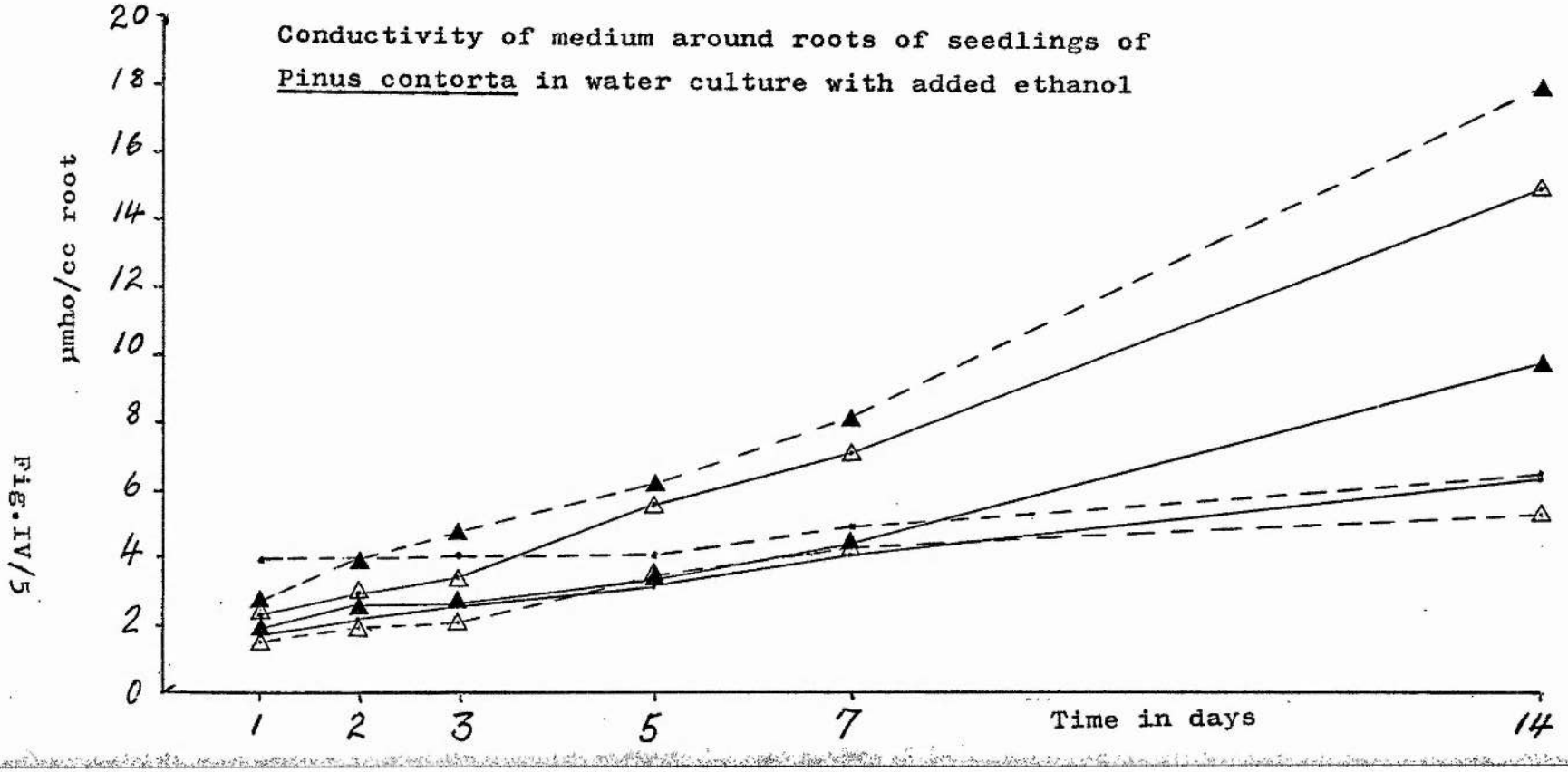

Conductivity of medium around roots of seedlings of
Pinus contorta in water culture with added ethanol
µmho/cc root
Time in days
0
2
4
6
8
10
12
14
16
18
20
1
2
3
5
7
14

Fig. IV/5

Fig. IV/6

Histograms of conductivity (μmho/cc root/day) of medium around roots of Pinus contorta seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of ethanol in the sealed vessels, as indicated.

Experimental Series C.

Each column represents the mean value for two culture vessels.

aerobic } no added ethanol
anaerobic }

10^{-4}M ethanol }
10^{-3}M ethanol } anaerobic
10^{-2}M ethanol }
10^{-1}M ethanol }

Conductivity of medium around roots of seedlings of Pinus contorta in water culture with added ethanol

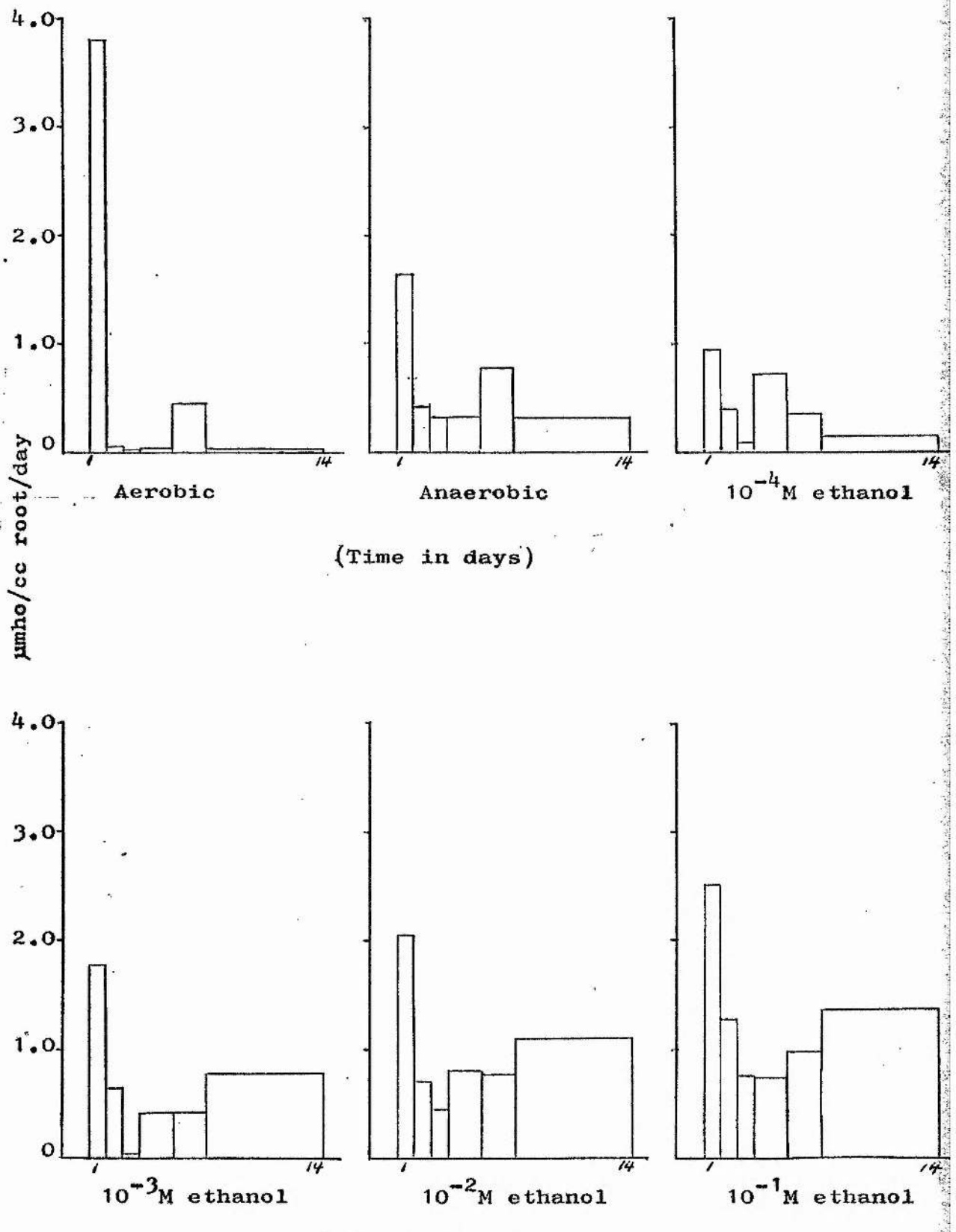


Fig. IV/6

Fig. IV/7

Conductivity (μmho/cc root) of medium around roots of Picea sitchensis seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

- - - aerobic
——— anaerobic
} no added ethanol

— ⊖ — 10^{-4}M ethanol
—●— 10^{-3}M ethanol
—⊖— 10^{-2}M ethanol
— ● — 10^{-1}M ethanol
} anaerobic

Each point represents the mean value for two culture vessels.

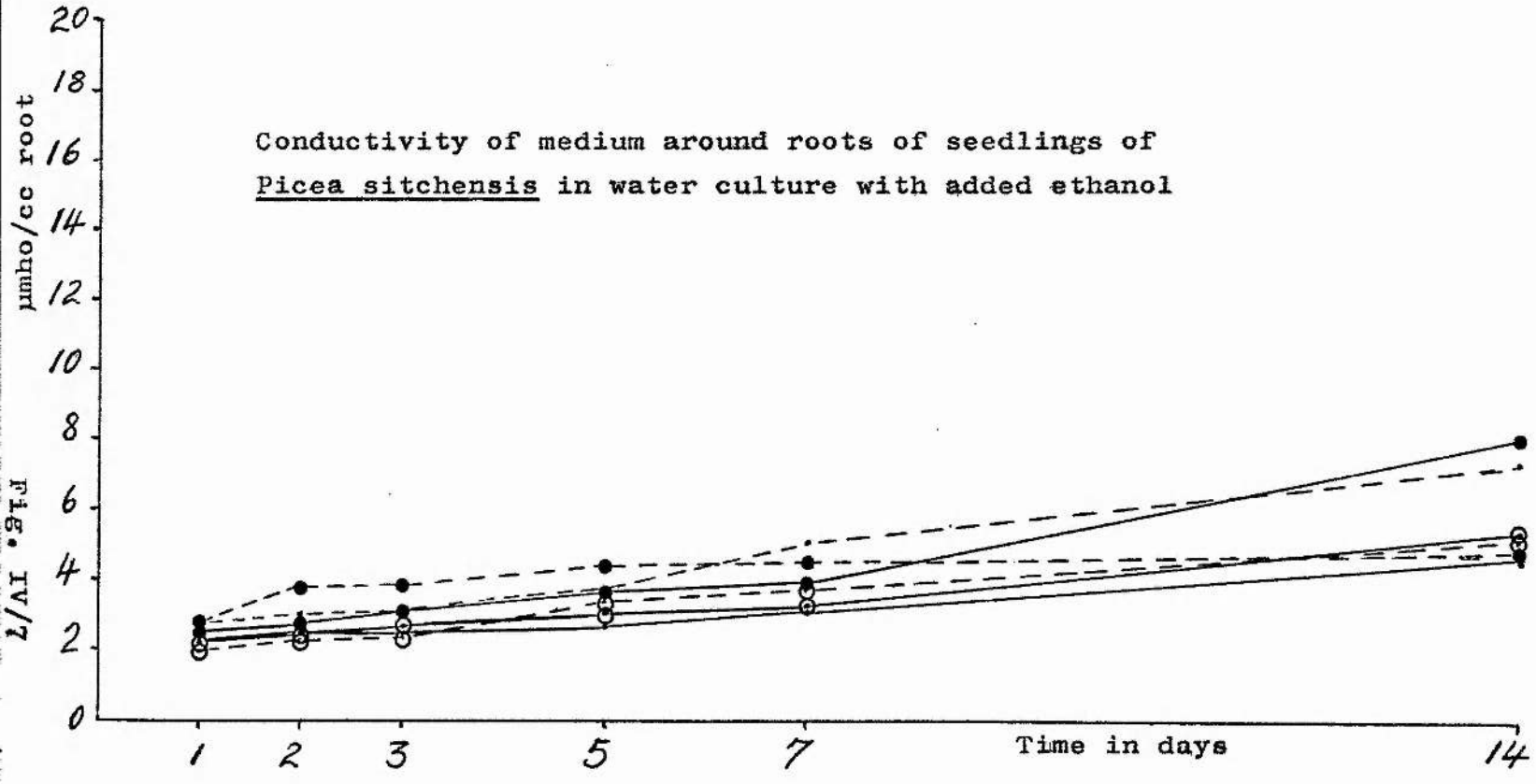

Conductivity of medium around roots of seedlings of
Picea sitchensis in water culture with added ethanol
µmho/cc root
Flg. IV/1
20
18
16
14
12
10
8
6
4
2
0
1
2
3
5
7
14
Time in days

Fig. IV/8

Histograms of conductivity (μmho/cc root/day) of medium around root of Picea sitchensis seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of ethanol in the sealed vessels as indicated.

Experimental Series C.

Each column represents the mean value for two culture vessels.

aerobic, anaerobic } no added ethanol

10^{-4}M ethanol, 10^{-3}M ethanol, 10^{-2}M ethanol, 10^{-1}M ethanol } anaerobic

Conductivity of medium around roots of seedlings of Picea sitchensis in water culture with added ethanol

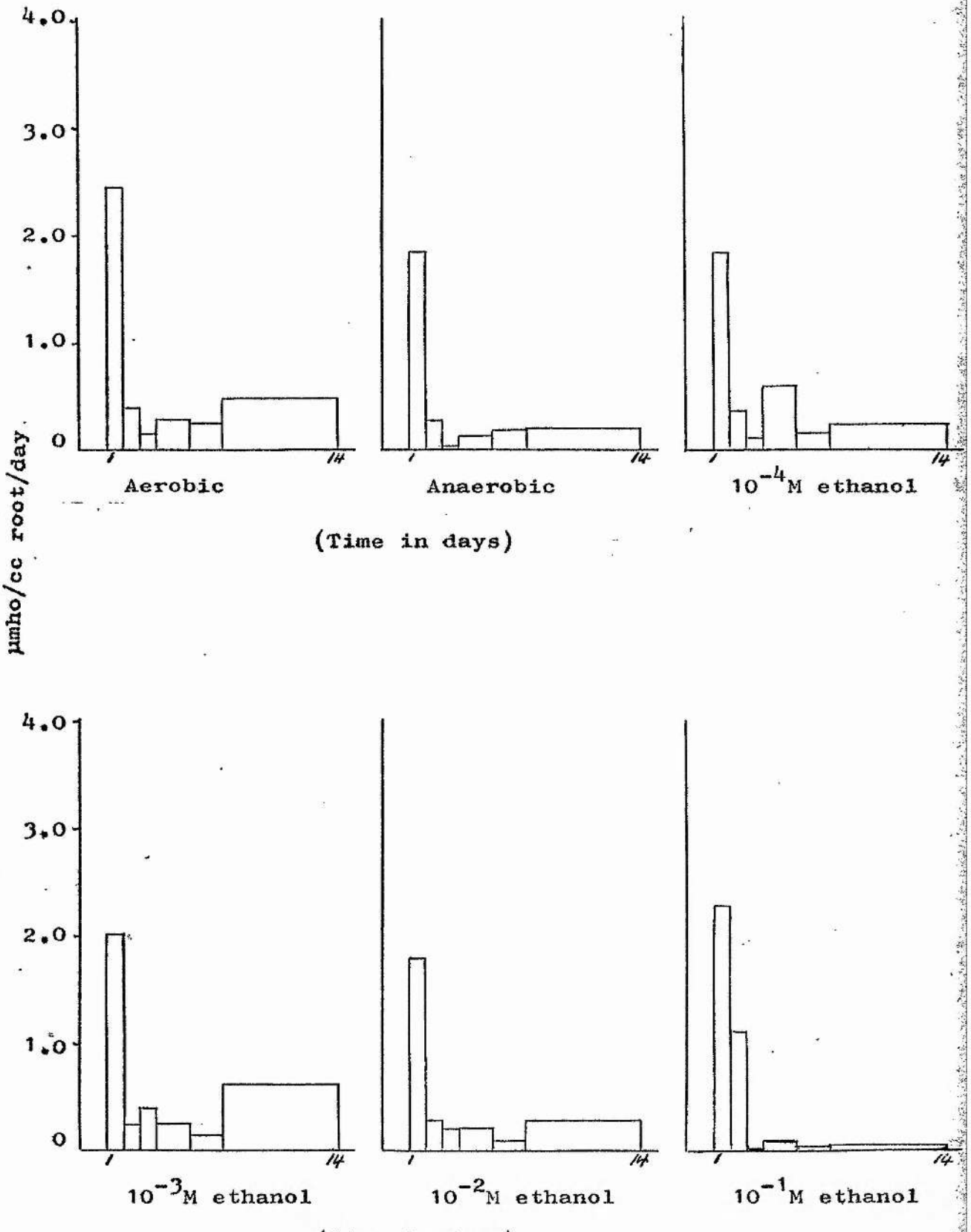


Fig.IV/8

Fig. IV/9

pH of medium around roots of Pinus contorta seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series A and C.

Treatments -

– ✱ –	aerobic Experimental Series A	no added ethanol
– • –	aerobic Experimental Series C	
—✱—	aerobic Experimental Series A	
—•—	anaerobic Experimental Series C	
– △ –	10^{-4}M ethanol	anaerobic Experimental Series C
—▲—	10^{-3}M ethanol	
—△—	10^{-2}M ethanol	
– •▲ –	10^{-1}M ethanol	

Each point represents the mean value for two cultures.

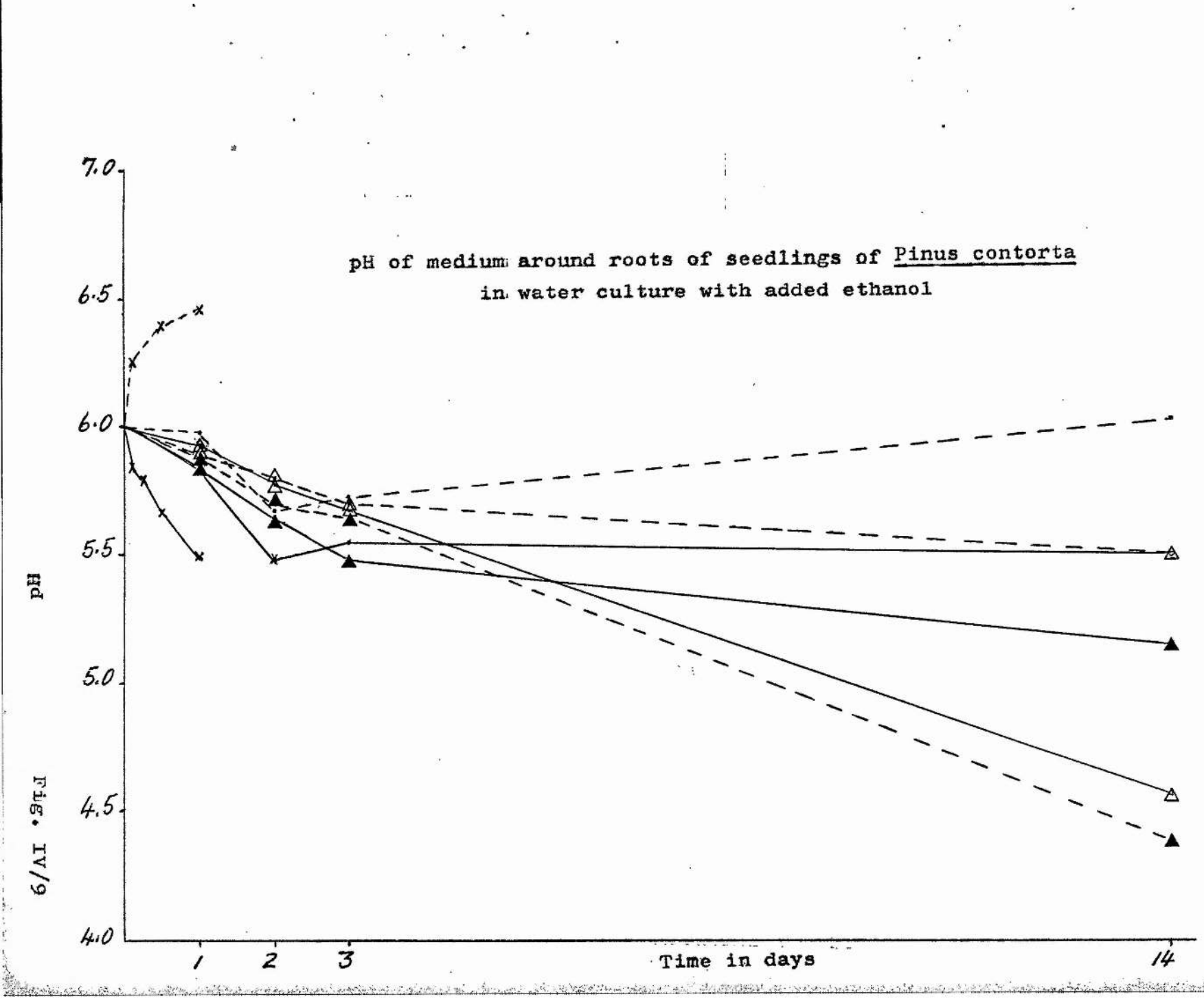

pH of medium around roots of seedlings of Pinus contorta
in water culture with added ethanol
7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
1
2
3
Time in days
14

Fig. IV/10

pH of medium around roots of Picea sitchensis seedlings in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels. Experimental Series A and C.

Treatment -

–*–	aerobic Experimental Series A	no added ethanol
–•–	aerobic Experimental Series C	
—×—	aerobic Experimental Series A	
—•—	anaerobic Experimental Series C	
–⊖–	10^{-4}M ethanol	anaerobic Experimental Series C
—●—	10^{-3}M ethanol	
—⊖—	10^{-2}M ethanol	
–●–	10^{-1}M ethanol	

Each point represents the mean value for two cultures.

pH of medium around roots of seedlings of Picea sitchensis
in water culture with added ethanol

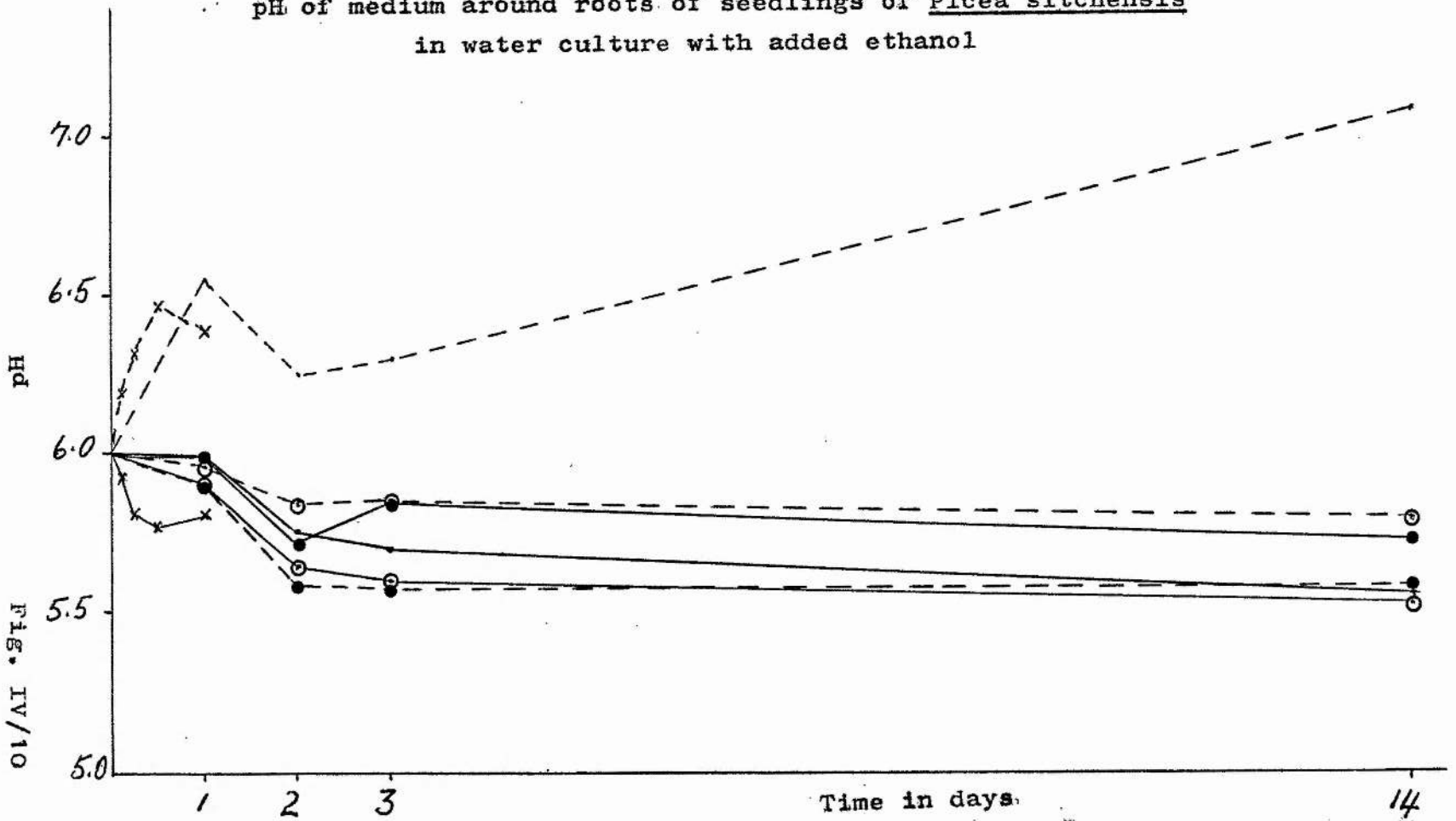


Fig. IV/10

See p. 59 for legend.

Fig. IV/11

Chlorophyll content of needles from seedlings of Pinus contorta in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

●	aerobic	no added ethanol
——	anaerobic	no added ethanol
–▽–	10^{-4}M ethanol	anaerobic
—▲—	10^{-3}M ethanol	anaerobic
—△—	10^{-2}M ethanol	anaerobic
–▲–	10^{-1}M ethanol	anaerobic

Each point represents the mean value for two cultures.

In aerated water culture, the pH of the media surrounding the roots of Pinus contorta seedling roots, (pH 6.4) does not reach as high a level as that surrounding Picea sitchensis seedling roots, pH 7.1.

The initial pH was pH 6.0.

2. B. Discussion

It is obvious from the results that the effect of exogenous ethanol is much more clearly defined in Pinus contorta than it is in Picea sitchensis and also that the parameters of leakage-potassium loss and conductivity - have much higher values in the media around Pinus contorta roots than Picea sitchensis roots. In all cases, the effects of different treatments did not become well differentiated until the experiment had proceeded for 14 days.

These findings are perhaps contrary to those expected if ethanol does have its toxic effect via the dissolution of membranes. It may have been expected that Pinus contorta, since it is flood tolerant, would be more resistant to this action of ethanol than Picea sitchensis. Alternatively, it may have been supposed that the two species would have approximately the same reaction to external ethanol. In this case, the higher flooding tolerance of Pinus contorta would derive simply from the fact established in the previous chapter, that this species produces less ethanol in its roots than Picea sitchensis in the same external, anaerobic conditions.

The results are, however, in accord with those found in Experimental Series A and Experimental Series C (Chapter I) where the effect of short term anaerobic root conditions on these parameters was investigated. Here too, the measurements for Picea sitchensis were lower than those for Pinus contorta, and showed no definite pattern, whereas those for Pinus contorta corresponded with both time and treatment. This underlines the difficulties continually encountered when trying to assess the reaction of Picea sitchensis roots to a given set of external root conditions.

It would seem that these results indicate that Picea sitchensis is more, rather than less resistant to the solvent action of ethanol on the root membranes. However, as suggested in the introduction to this section, there are several causes of leakage, and it may be that another, other than the solvent action of ethanol is the main one operative in this case. If, as suggested in Chapter 1, active excretion rather than passive leakage, is a major factor controlling the loss of substances from the roots (Grineva 1962), then any effect that ethanol had on the energy metabolism of the roots, would be reflected in the decrease of actively excreted material in the medium around the root. The consequent increase in the concentration of the cytoplasm may itself result in damage to the root. However, as previously remarked, it would seem to be rather a paradox for a widely recognised symptom of anaerobic root conditions, to be one that requires the presence of oxygen to become apparent. The alternative proposed - that Picea sitchensis is more sensitive to the mechanical effect of forced aeration seems more likely.

The observation that, in both species to some extent, the pH of the root medium decreases in anaerobic conditions, is probably due to an increase in the loss of organic and amino acids. Since it appears that the leakage of substances from Picea sitchensis roots is less overall than that from Pinus contorta, and that the pH in anaerobic conditions is higher (less acidic), it seems feasible that this is due to a smaller loss of organic and amino acids from the roots.

Although low pH is known to enhance solute loss (Christiansen et al 1970; Rains et al 1964), it is probable that the levels attained in these experiments are not usually high enough to induce marked H ion damage. However, Marschner (1966) did find that corn root tips suffer considerable damage to fine structure, and an increase in potassium loss at pH 4.4. Since the medium surrounding the roots of Pinus contorta in 10^{-2} and 10^{-1}M ethanol reached a pH of 4.56 and 4.38 respectively, after 14 days, there is still a possibility of H ion damage. The higher pH levels found in aerated treatments probably arise from some loss of cations.

The results presented in this section still do not give very clear indications as to the cause of leakage in anaerobic conditions. However it is improbable that active excretion and H ion damage play very major roles in leakage, and it is likely that what takes place is a combination of the effects of reduced energy, and increased ethanol. Energy levels are reduced simply because the output per mole of glucose is lower in anaerobic than aerobic respiration. However, the reduced energy level may also be linked with the presence of ethanol, since the ethanol in the cells may start to disrupt the membranes of various organelles such as mitochondria, and by this action will interfere with aerobic respiration and reduce the level of ATP in the cell. This reduction in energy charge induces a Pasteur Effect, resulting in the production of more energy, but also of even more ethanol.

3. VIABILITY OF ROOT TIPS IN ETHANOL

Whatever its mode of action, if ethanol proves lethally toxic to roots, its actions may be monitored by observing the viability of root tips which have been immersed in ethanol. Any effect would be expected to increase with the concentration of ethanol. Chirkova (1975) has found that the roots of willow, a flood tolerant tree, remain viable in the 10^{-1}M ethanol after 6 days, whereas those of Poplar, a non-flood tolerant tree, are killed by 2 days of immersion.

Root tips from plant roots which had been immersed in a range of unaerated ethanol solutions (or water) were observed using tetrazolium stain. (Experimental Series E)

3. A. Results

Species	Conditions	Description	
		1 day	7 days
Picea sitchensis	water	Tips alive. Central area of tip stained. Some dead tips.	Pinkish stain in cortex, not in central region.
	2×10^{-2}M ethanol	Tips mostly alive, stele stained in some.	Some tips with pink stained stele. Most dead.
	2×10^{-1}M ethanol	"	Outside layers brown, also stele in some. Most dead.
	2M ethanol	Most roots dead.	Roots dead and disintegrating.
Pinus contorta	water	Tips alive. Central area of tip stained deep red.	Most tips red, but some dead. New roots present - thick and white.
	2×10^{-2}	"	Tips of new roots red. Most others dead.
	2×10^{-1}	Most tips alive, some dead.	Outside layers brown in many roots, also stele in some.
	2M ethanol	Most roots dead.	Roots mostly dead and disintegrating.

3. B. Discussion

In both species, the effect of 2M ethanol is seen to be complete and fairly rapid; most roots being dead after one day of immersion. However, the effects of the other concentrations seem more marked in Picea sitchensis than in Pinus contorta after 7 days immersion. Whilst in most cases many of the root tips were dead or dying in Pinus contorta new roots were also present on plants with roots immersed in 2×10^{-2}M ethanol. Vester (1972) has previously noted that Pinus contorta seedlings can only survive waterlogged conditions undamaged for more than 8 months, if they are able to form adventitious roots on the soil surface, and that this ability is only maintained in seedlings of flood tolerant species.

Tomato plants also produce adventitious roots near the surface, under flooding conditions (Waddington and Baker 1965), and if these are not formed, severe chlorosis ensues (Jackson 1956). Kramer (1951b) found that sunflower plants, which were the least damaged by flooding, of the species he observed, were also the only ones which had produced adventitious roots. Pelargonium, Impatiens and Phaseolus plants subjected to aerated water culture produce adventitious and are then able to withstand subsequent unaerated water culture (Bergman 1920). Kramer (1951b) suggested that the new roots produced may differ anatomically and/or physiologically from the old roots. Some workers (Luxmore and Stolzy 1969; Yu, Stolzy and Letey 1969) have found that adventitious roots produced as a result of anaerobic conditions are more porous than normal roots. Kramer (1951b) however, states that, although these new roots do have large intercellular spaces, thinner cell walls, and less suberisation, there is no direct evidence that these characters provide a more adequate supply of air to the roots (Gill 1975). Even in cases where diffusion of oxygen from the shoot to the root and even out of the root have been demonstrated (Armstrong 1967; Armstrong 1968; Krasovskii and Chashchukhin 1974) this does not necessarily mean that the oxygen is in excess of the root requirements.

As mentioned in Chapter II, oxygen diffusion from the roots cannot be equated with tolerance of flooding (Greenwood 1967; Jensen et al 1964). It is known (Erickson 1965; Grineva 1963; Letey, Stolzy and Blank 1962) that reduced oxygen in the root zone can be more detrimental if it occurs during an early stage of growth before a good root system has developed. It may be that species which are able to produce new, perhaps adaptive roots (this may simply be in position, e.g. at the soil surface), before the old roots are killed, are better able to survive periods of flooding. This may include both the ability to produce these new roots quickly, and also the ability to maintain the viability of the old roots long enough for this production to occur. Picea sitchensis may possibly have the ability to produce adaptive roots, but may not have the ability to produce them

quickly enough, or to maintain the viability of the older roots long enough.

This may be one instance where the ability to maintain longer periods of root viability, but the regulation of ethanol production, could play an important role.

4. CHLOROPHYLL CONTENT OF NEEDLES

If exogenous ethanol has a marked effect on the roots, this should be reflected in the physiological state of the aerial organs. The effect of short term anaerobic root conditions on leaf chlorophyll could not be satisfactorily detected in Experimental Series B (Chapter I). This present investigation (Experimental Series C) was of increased duration, and included ethanol treatments (as well as the unaerated water culture previously discussed in Chapter I).

4. A. Results

In all cases, it was evident that the varying ethanol concentration exerted different affects. However, unlike the results shown for leakage, these effects are more marked in Picea sitchensis than in Pinus contorta. It is probable that results expressed as mg/mm^2 give a more accurate picture of the chlorophyll content than results expressed as mg/g (fresh wt.). This is because, during flooding, there is a decrease in water absorption (Kramer 1951b), and a subsequent reduction in the water content, and hence the fresh weight of the leaves. The area of the leaves, however, remains relatively unchanged.

The graph of total chlorophyll content expressed as mg/mm^2 (fig. IV/11) shows that, in Pinus contorta most plants, after an initial decline, seem to resume normal chlorophyll levels (perhaps even heightened to some extent), except for those with roots immersed in 10^{-1}M ethanol. These plants suffer a continued decline in total chlorophyll after 14 days, 10.59 mg/mm^2, compared to 16.99 mg/mm^2 as found in leaves from plants in aerated water culture. This pattern is also reflected in the levels of chlorophyll-a, although it appears that the levels of

Chlorophyll content of needles from seedlings of Pinus contorta in water culture with added ethanol

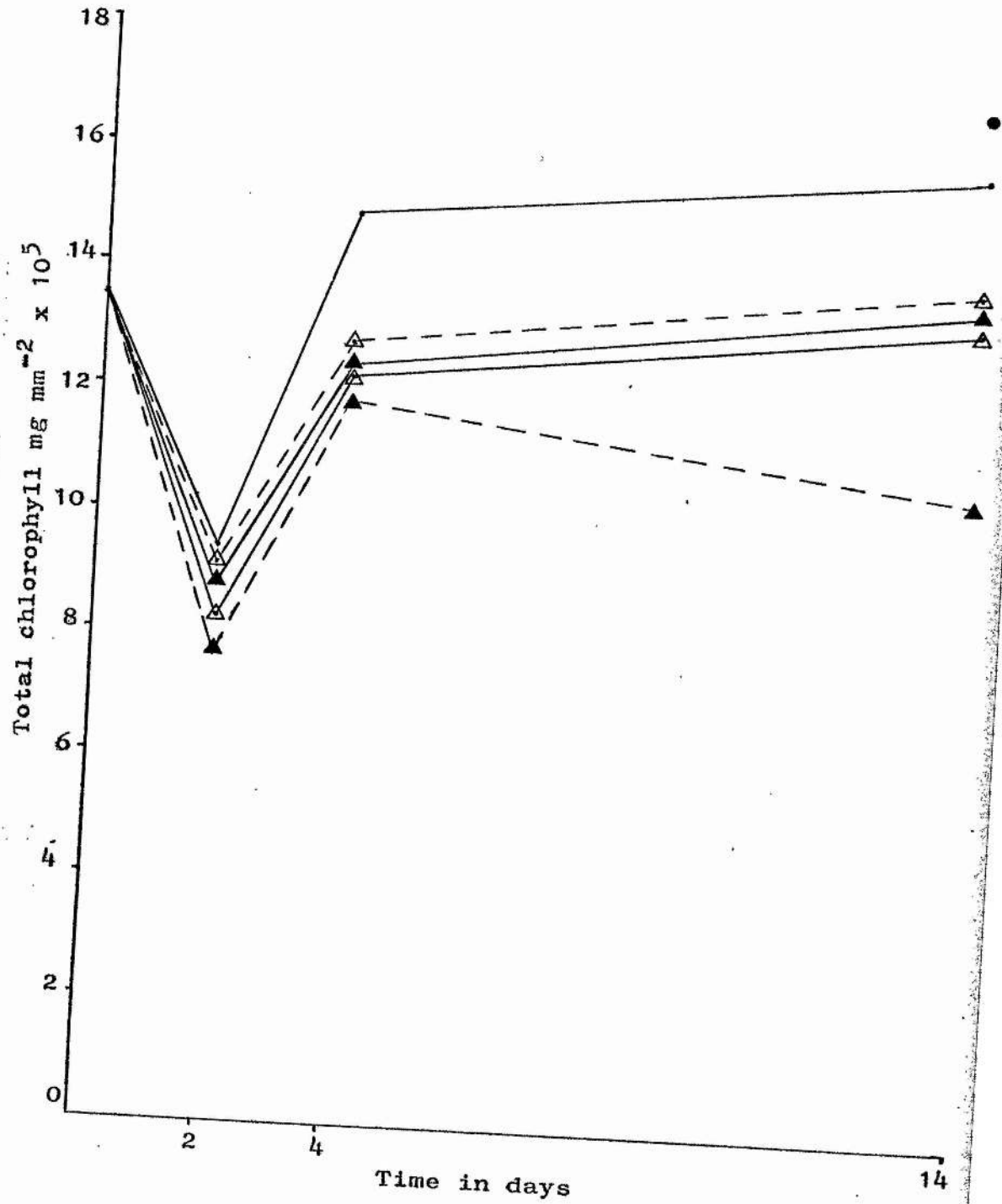


Fig. IV/11

Fig. IV/12

Chlorophyll a and Chlorophyll b content of needles from seedlings of Pinus contorta in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

o	aerobic	no added ethanol
——	anaerobic	
–△–	10^{-4}M ethanol	anaerobic
—▲—	10^{-3}M ethanol	
—△—	10^{-2}M ethanol	
–▲–	10^{-1}M ethanol	

Each point represents the mean value for two culture vessels.

Chlorophyll a and Chlorophyll b
content of needles from seedlings
of Pinus contorta in water culture
with added ethanol

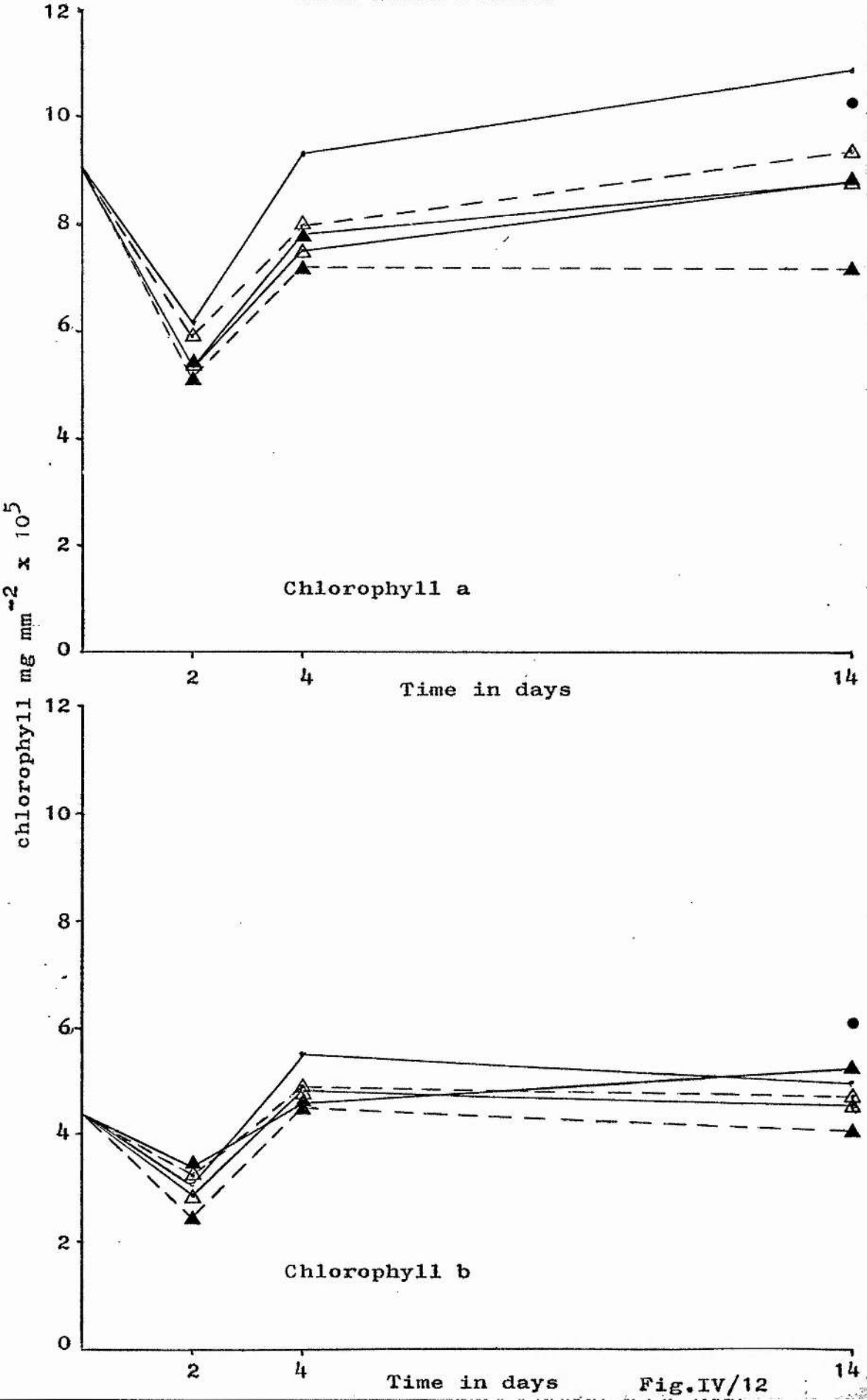


Fig.IV/12

Fig. IV/13

Chlorophyll content of needles from seedlings of Picea sitchensis in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

■	aerobic	no added ethanol
———	anaerobic	
-⊖-	10^{-4}M ethanol	anaerobic
—⊖—	10^{-3}M ethanol	
—⊖—	10^{-2}M ethanol	
-⊖-	10^{-1}M ethanol	

Each point represents the mean value for two cultures.

Chlorophyll content of needles from seedlings of Picea sitchensis in water culture with added ethanol

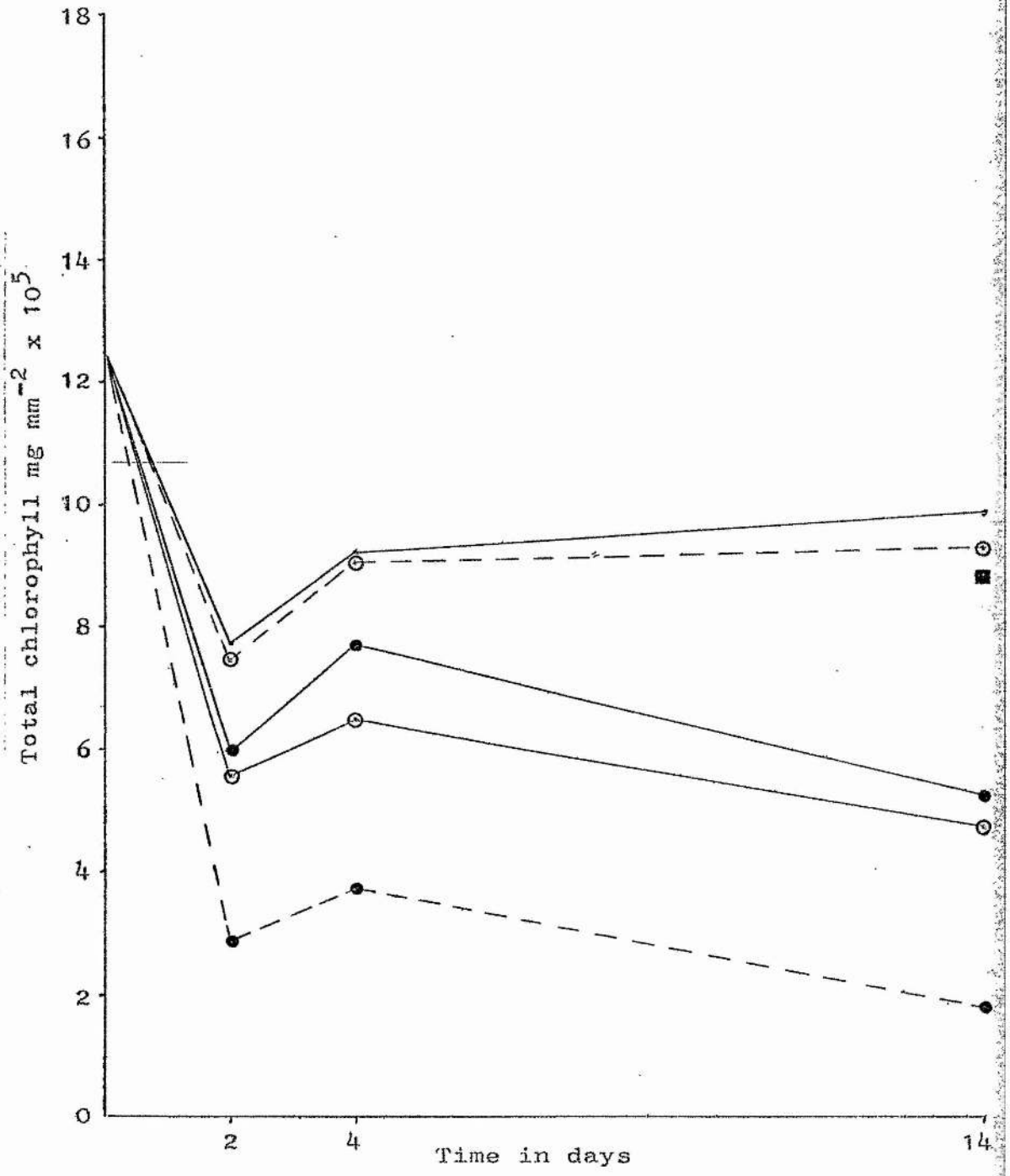


Fig. IV/13

Fig. IV/14

Chlorophyll a and Chlorophyll b content of needles from seedlings of Picea sitchensis in water culture in unsealed and aerated (aerobic) or sealed (anaerobic) 600 ml vessels, with or without various concentrations of added ethanol in the sealed vessels.

Experimental Series C.

Treatments -

■	aerobic	no added ethanol
———	anaerobic	no added ethanol
– ⊖ –	10^{-4}M ethanol	anaerobic
—●—	10^{-3}M ethanol	anaerobic
—⊖—	10^{-2}M ethanol	anaerobic
– ● –	10^{-1}M ethanol	anaerobic

Each point represents the mean value for two culture vessels.

Chlorophyll a and Chlorophyll b content of needles from seedlings of Picea sitchensis in water culture with added ethanol

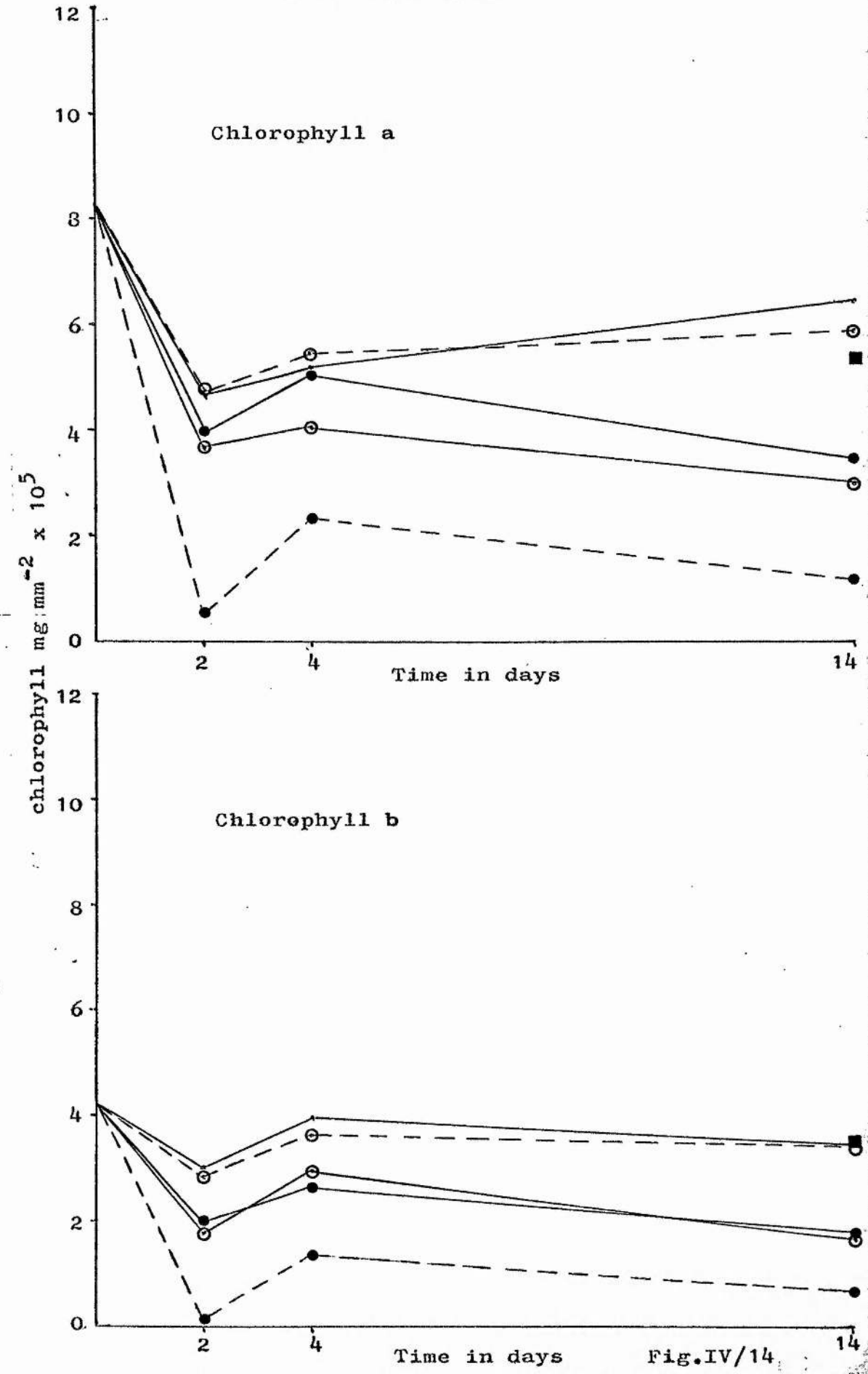


Fig.IV/14

chlorophyll-b do not fluctuate with quite the same amplitude (fig. IV/12).

The differential effects of different ethanol concentrations on chlorophyll levels, is much more marked for Picea sitchensis (fig. IV/13). The lowest level reached is in leaves from plants with roots immersed in 10^{-1}M ethanol, after 14 days, 1.07 mg/mm^2. The maximum value attained is in plants in unaerated water culture after 14 days. Here the chlorophyll levels reach 9.93 mg/mm^2, a figure still below the minimum recorded for Pinus contorta.

The results for Picea sitchensis seedlings in aerated water culture do not agree with the general trend as observed in Pinus contorta and the remainder of the Picea sitchensis results; that is, they do not follow the pattern of a decline in chlorophyll levels (after 14 days), from a maximumobserved in aerated culture, through a lower level in unaerated culture, and through increasingly lower levels in the greater ethanol concentrations, until a minimum is reached in the leaves from those plants having their roots immersed in 10^{-1}M ethanol. In the case of Picea sitchensis, plants in aerated water culture do not regain their normal chlorophyll level after 14 days treatment, rather, the level remains low, at 8.78 mg/mm^2, comparable to that found in unaerated water culture and in 10^{-4}M ethanol treatments. Unlike Pinus contorta there does not, in Picea sitchensis, seem to be any substantial recovery of chlorophyll level after the initial decline, in any treatment. Levels are generally lower still after 14 days (as expressed in mg/mm^2). Chlorophyll-a levels reflect this pattern, but, as in Pinus contorta, Chlorophyll-b levels do not fluctuate so greatly, although they do again follow the same pattern. (fig. IV/14).

4. B. Discussion

These results are interesting in that they show that ethanol applied exogenously to the roots can exent influence on chlorophyll levels in the leaves. It is also interesting to note that whilst Pinus contorta exhibited higher levels of leakage when roots were treated with ethanol, than did Picea sitchensis, in

this instance, the opposite reaction is true, viz, Pinus contorta showed less effect of added ethanol on chlorophyll levels than did Picea sitchensis. Once more, however, Picea sitchensis in aerobic conditions was more detrimentally effected than Picea sitchensis in anaerobic and even some ethanol treatments.

It has been considered in the past (Ferguson and Simon 1973; Kramer 1951b) that leaf yellowing and death during flooding could be caused by desiccation. However, as Kramer himself points out, there could also be transport of toxic substances up the stem from dying roots. The occurrence of ethanol transport up the stem has been demonstrated by several workers. Huck and Erickson (unpublished) have found ethanol present in the xylem sap of tomato, cotton, and soybean plants, Leblova et al (1969) found that it was transported to leaves of pea plants, and Kenefick (1962) has shown that sugar beet leaves can in fact transpire ethanol supplied externally to the roots. Chirkova (1975) has also shown this using C^{14} ethanol, but she states that the ethanol produced is subsequently metabolised (see also Coussins & Beevers 1963; Taylor 1942), and so it may be ethanol itself or products of its metabolisation which are transported to the overground organs. Her experiments indicated that ethanol, under conditions of aeration, was more actively implicated in metabolism of willow tissue (roots and leaves), than it was in the tissue of the non flood tolerant poplar. The label was absent in the carbohydrate fractions from roots and leaves of poplar, suggesting that sugars were not, at that stage, being synthesised. This may have been connected with a decrease in photosynthesis due to the effect of ethanol on the leaves.

Khotianovich (1958), during an investigation on the effects of flooding on pines, found that here the amount of chlorophylls a and b in the needles, was 15% smaller than the content found for pines growing in normal conditions, and he concludes that the synthesis of many important compounds, including chlorophyll, in the aerial organs of trees, and in particular, in the needles,

depends to a great degree, on reactions occurring in the root system of the tree. Baltcheffsky (1963), has noted that lower aliphatic straight chain alcohols inhibit photophosphorylation in isolated spinach chloroplasts, and considers that this effect is probably due to the interferance of alcohol molecules in the chloroplast membranes. He states that alcohol "may cause inhibition by changing, through mass action, the three dimentional geometrical structure of the photosynthetic apparatus".

It seems likely that, if alcohol is transported to the leaves, and exerts some toxic influence, then the nature of this action is probably the same as that which is occurring in the roots.

5. ROOT RESPIRATION

Harris and Van Bavel (1957, cited by Grable 1966), have said that root respiration is "the most sensitive aspect of plant activity in regard to soil aeration" and "it may be assumed that reduction in respiratory activity is the first step in growth limiting effects of insufficient aeration". This latter statement may or may not be true; if a low level of anaerobic respiration is accompanied by a low accumulation of ethanol, this may in fact be advantageous to the plant. In any event, in a study of the effect of flooding on root metabolism, the reaction of the root respiration to conditions of anoxia must be of interest. This study also includes the reaction of root respiration to externally supplied ethanol, in both aerobic and anaerobic conditions. Unlike the previous experiments, anaerobic conditions were achieved in this experiment by bubbling with nitrogen gas. This means that any mechanical effects of bubbling are equally present in all treatments, and the effects of aerobic and anaerobic conditions may be compared apart from any effect of this bubbling. (Experimental Series F.).

5. A. Results

AEROBIC RESPIRATION: In both species, the QO_2 is found to be higher in those plants pretreated aerobically than in those pretreated anaerobically (water culture, no added ethanol (fig. IV/15). Addition of ethanol to the root medium has the effect of increasing QO_2 of those plants of both species pretreated aerobically , (fig. IV/17). The stimulatory effect increases with ethanol concentration. In anaerobically treated plants, ethanol has a stimulatory effect on the QO_2 of Pinus contorta roots up to a concentration of 10^{-2}M ethanol, QO_2 is then lower again (though not as low as normal water culture anaerobic levels) at 10^{-1}M ethanol. QO_2 is in all cases lower for Pinus contorta roots pretreated anaerobically, despite any ethanol treatment, than it is for Pinus contorta roots pretreated aerobically.

The effect of different concentrations of ethanol on roots of anaerobically grown Picea sitchensis plants is rather more erratic (see histogram, fig. IV/17). However, apart from a high QO_2 recorded for roots in 10^{-2}M ethanol, in general, added ethanol depresses the value of QO_2 below that found in anaerobically treated roots with no added ethanol. This of course, is in contrast with the findings in Pinus contorta.

Despite the apparent depression of QO_2 by ethanol in Picea sitchensis plants treated anaerobically, the level of QO_2 in this treatment for roots having no ethanol treatment, is only slightly less than that found for roots pretreated aerobically; 0.59, compared to 0.64. For Pinus contorta, this ratio is 0.74 to 0.39 - the anaerobically pretreated roots have a much lower QO_2 in this case (fig. IV/15).

QCO_2 levels mirror those for QO_2 in most cases, although there are a few exceptions, for example, QCO_2 for roots of Pinus contorta pretreated anaerobically, drops at 10^{-1}M instead of increasing still further as is the case with QO_2. The most marked difference occurs between roots of both species with no added

ethanol. In the case of Pinus contorta, QCO_2 is higher in plants pretreated aerobically than in those plants pretreated anaerobically (0.76 to 0.33). In Picea sitchensis, QCO_2 is lower in plants pretreated aerobically than it is in plants pretreated anaerobically, (0.52 to 0.57), although, as is the case with QO_2, these values are much closer together than those found in Pinus contorta.

RQ is depressed by anaerobic pretreatment in Pinus contorta, whereas it is increased by this treatment in Picea sitchensis (fig. IV/15). Despite this, RQs are lower overall in anaerobically pretreated plants of both species, when ethanol treatment is taken into account (fig. IV/19). In aerobically pretreated Pinus contorta, RQ appears to remain remarkably stable regardless of ethanol treatment (apart from the value obtained for roots in 10^{-3}M ethanol). The values are again steady, though lower, for anaerobically pretreated Pinus contorta, In this case, ethanol appears to cause some raising of RQ above the normal anaerobic level.

The RQ of Picea sitchensis roots grown aerobically is increased by ethanol concentrations up to 10^{-2}M, but remains at the same level as "no added ethanol" in 10^{-1}M ethanol. The situation in anaerobically pretreated plants is far from clear, but there seems to be an overall depression of RQ by added ethanol, especially marked at 10^{-1} M ethanol, where the RQ is far below that found when no ethanol is added.

ANAEROBIC RESPIRATION: In both species the values attained for $QCO_2(N_2)$ are found to be lower for those roots which had been pretreated anaerobically, than those treated aerobically (no ethanol)(fig. IV/16). Once again, the difference between the two values is not very marked in Picea sitchensis - 0.32 aerobic, compared to 0.22 anaerobic; whereas it is well marked in Pinus contorta - 0.51 compared to 0.19. Thus, although the level of CO_2 production in nitrogen is higher for aerobically pretreated Pinus contorta than for similarly treated Picea sitchensis, the CO_2 production in nitrogen for anaerobically pretreated

plants is lower in Pinus contorta than in Picea sitchensis. This can be seen more clearly by looking at the ratios of CO_2 production in nitrogen for the two pretreatments (table IV/A).

Low concentrations of ethanol have a stimulatory effect on anaerobic CO_2 production in both species and pretreatments. Higher concentrations of ethanol (10^{-2}M and 10^{-1}M) have a depressing effect in all cases except anaerobically pretreated Pinus contorta. (fig. IV/20).

The Pasteur Effect ($\frac{3xQCO2(N2)}{QCO2\ air}$), is found to be higher in both species in those roots pretreated aerobically (no ethanol - fig. IV/16). In this case, the difference between values in the different pretreatments is more marked in Picea sitchensis than in Pinus contorta. In Picea sitchensis, the values are 1.70 for the aerobic pretreatment, and 1.08 for the anaerobic pretreatment. For Pinus contorta the figures are 2.05 for the aerobic pretreatment, and 1.85 for the anaerobic pretreatment. Apart from a raised level observed for Pinus contorta roots in 10^{-4}M ethanol, increasing ethanol concentration have a depressing effect on P.E. in both species when pretreated aerobically (fig. IV/21). This effect is more dramatic in Picea sitchensis at the higher concentrations. Increasing ethanol concentration appear to affect the two species differently when they are pretreated anaerobically. In Pinus contorta the effect is not clear, causing a reduction in P.E. at 10^{-4}M and 10^{-2}M ethanol, but none at 10^{-3}M ethanol. 10^{-1}M ethanol increases the Pasteur Effect. For Picea sitchensis pretreated anaerobically, low concentrations of ethanol appear to have a dramatic effect, increasing the P.E. over two fold above the "no ethanol" level, when roots are in 10^{-4}M ethanol. Higher concentrations have a lowering effect on P.E. however.

VISUAL DESCRIPTION OF TREES AT END OF EXPERIMENTAL SERIES F:

Pinus contorta aerobic - healthy root and shoot in no ethanol and 10^{-4} ethanol treatments. Some new roots present in 10^{-4}M, and 10^{-3}M ethanol, although some of the older roots were moribund in 10^{-3}M ethanol. Some roots also moribund in 10^{-2}M ethanol. Roots appear dead in 10^{-1}M ethanol, though shoot healthy.

Picea sitchensis aerobic - shoot somewhat epinastic, and some of roots moribund in no ethanol, 10^{-4}M and 10^{-3}M ethanol. Some new roots present in 10^{-3}M ethanol. Shoot branches very epinastic and roots appearing dead in 10^{-2} and 10^{-1}M ethanol.

Pinus contorta anaerobic ($+N_2$) - shoot healthy and root rather moribund in most treatments, though some new roots present in 10^{-3}M ethanol treatment. General increase in root death with ethanol concentration.

Picea sitchensis anaerobic ($+N_2$) - shoot branches epinastic and roots rather moribund in most treatments, some shoots in higher ethanol concentrations rather desiccated, and leaf fall occurred in all ethanol treatments.

$QCO_2(N_2)$

	ratio - air pretreatment: Nitrogen pretreatment	
	Lodgepole pine	Sitka spruce
No ethanol	1:0.3673	1:0.6792
10^{-4}M ethanol	1:0.3735	1:0.4744
10^{-3}M ethanol	1:0.5804	1:0.6661
10^{-2}M ethanol	1:0.5749	1:0.8315
10^{-1}M ethanol	1:0.7741	1:0.6436

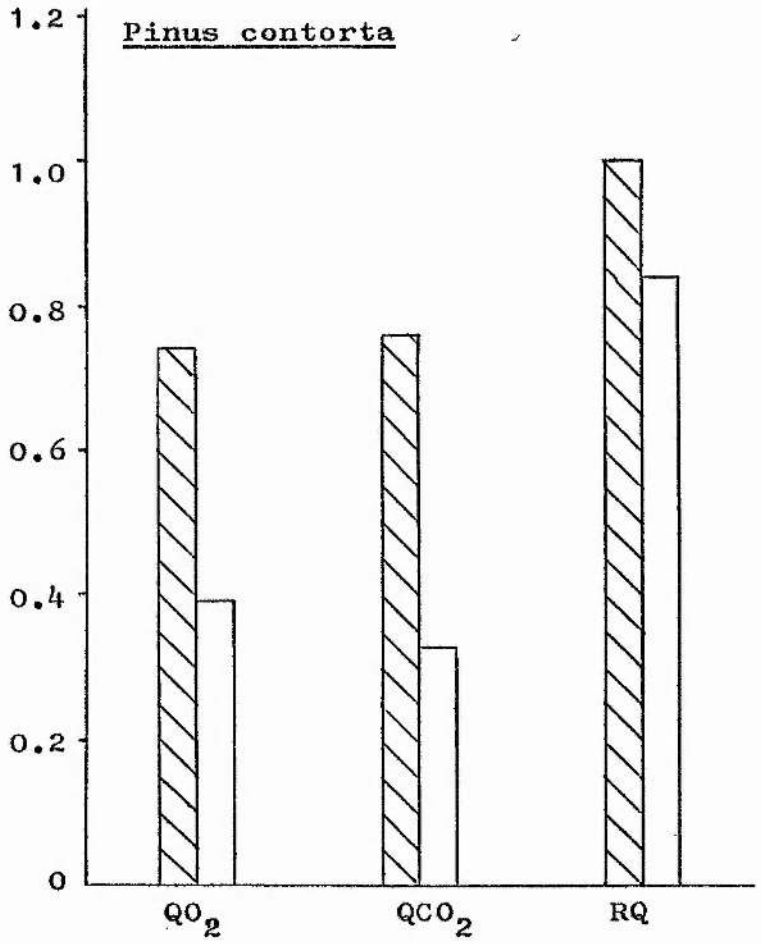
TABLE IV/A

Fig. IV/15

Respiration, in terms of QO_2, QCO_2 (in μl/h/mg dry wt) and RQ, of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns).

Each column represents the mean value of 2 separate determinations from roots of two seedlings.

Respiration of roots from tree seedlings in aerobic or anaerobic water culture



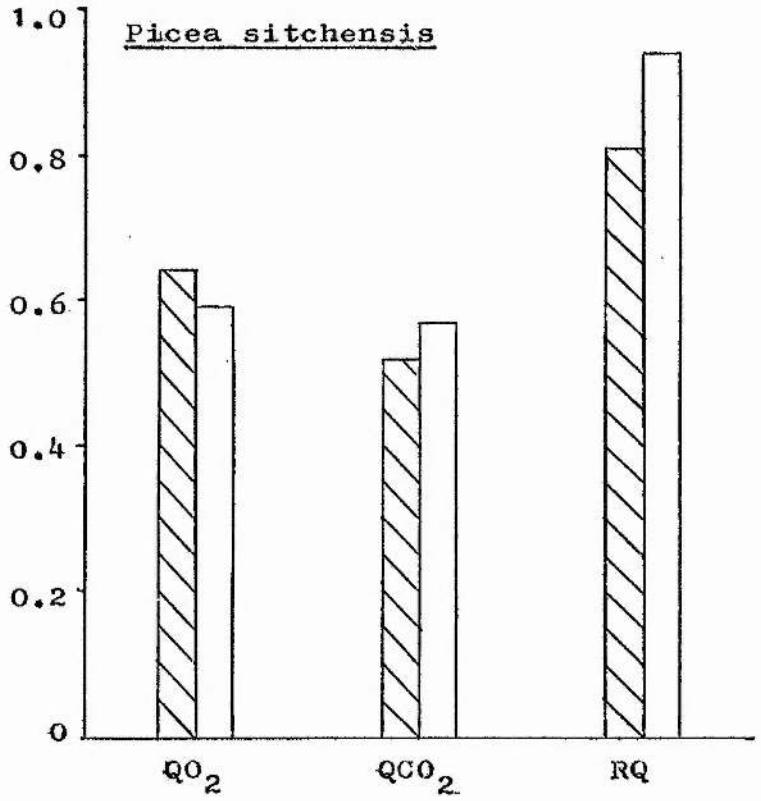


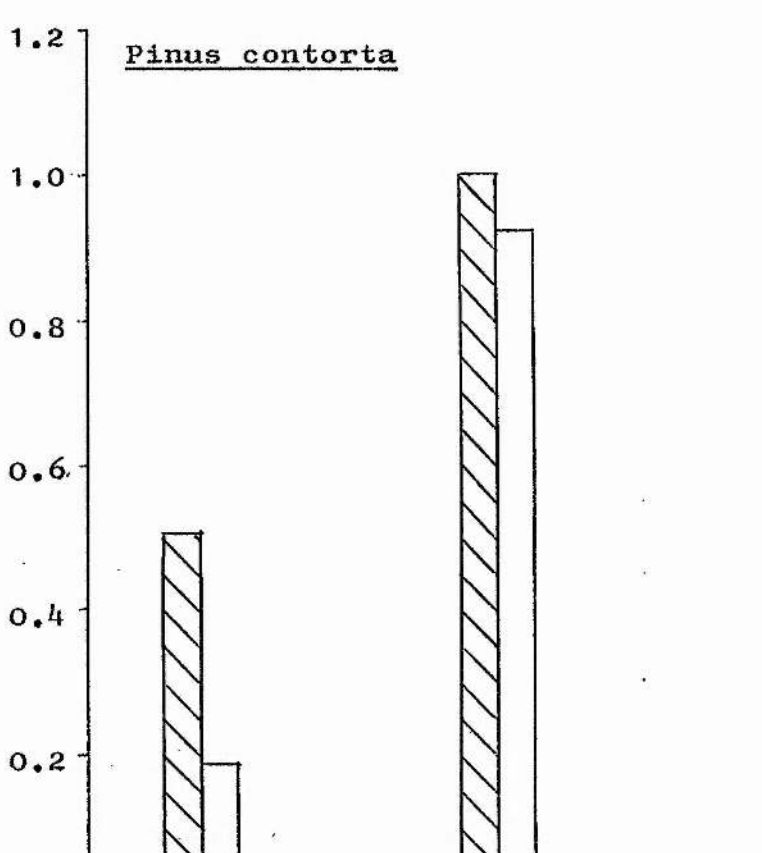
Fig. IV/15

Fig. IV/16

Anaerobic respiration, in terms of $\mathcal{Q}CO_2(N_2)$ (in μl CO_2 evolved/h/mg dry wt) and P.E. (Pasteur Effect, see text) of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns).

Each column represents the mean value of 2 separate determinations from roots of two seedlings.

Respiration of roots from tree seedlings in aerobic or anaerobic water culture



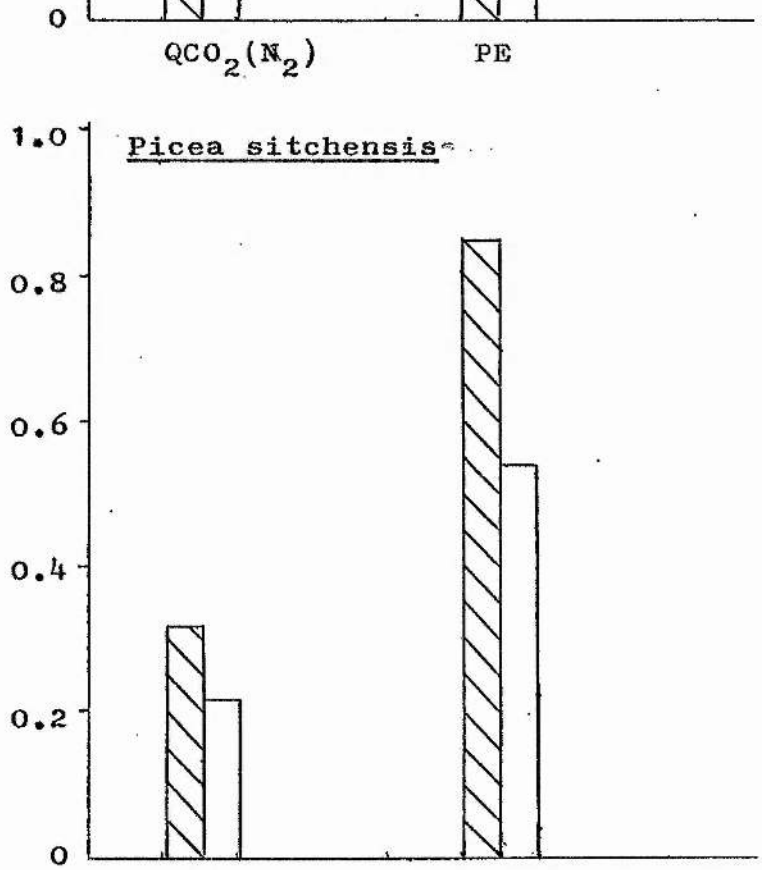


Fig. IV/16

Fig. IV/17

Oxygen uptake, QO_2, (in µl/h/mg dry wt) of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre vessels (Experimental Series F.). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns). Culture vessels also contained added ethanol at various concentrations as indicated.

1 - no added ethanol

2 - 10^{-4}M ethanol

3 - 10^{-3}M ethanol

4 - 10^{-2}M ethanol

5 - 10^{-1}M ethanol

Each column represents the mean value of two separate determinations from roots of two seedlings.

QO_2 of roots from tree seedlings
in water culture with added ethanol

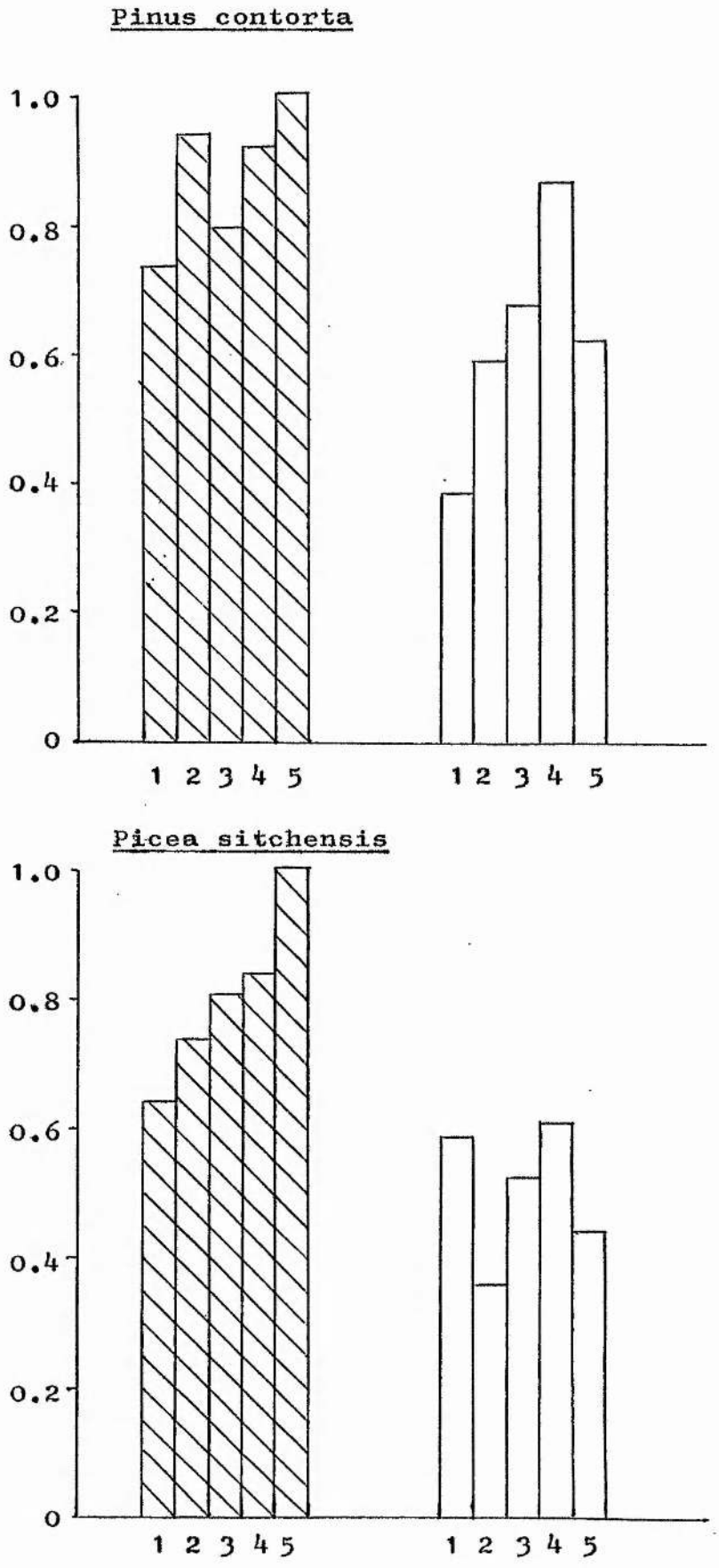


Fig. IV/17

Fig. IV/18

Carbon dioxide evolution, QCO_2, (in µl/h/mg dry wt) of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns). Culture vessels also contained added ethanol at various concentrations as indicated.

1 - no added ethanol
2 - 10^{-4} ethanol
3 - 10^{-3}M ethanol
4 - 10^{-2}M ethanol
5 - 10^{-1}M ethanol

Each column represents the mean value of two separate determinations from roots of two seedlings.

QCO_2 of roots from tree seedlings in water culture with added ethanol

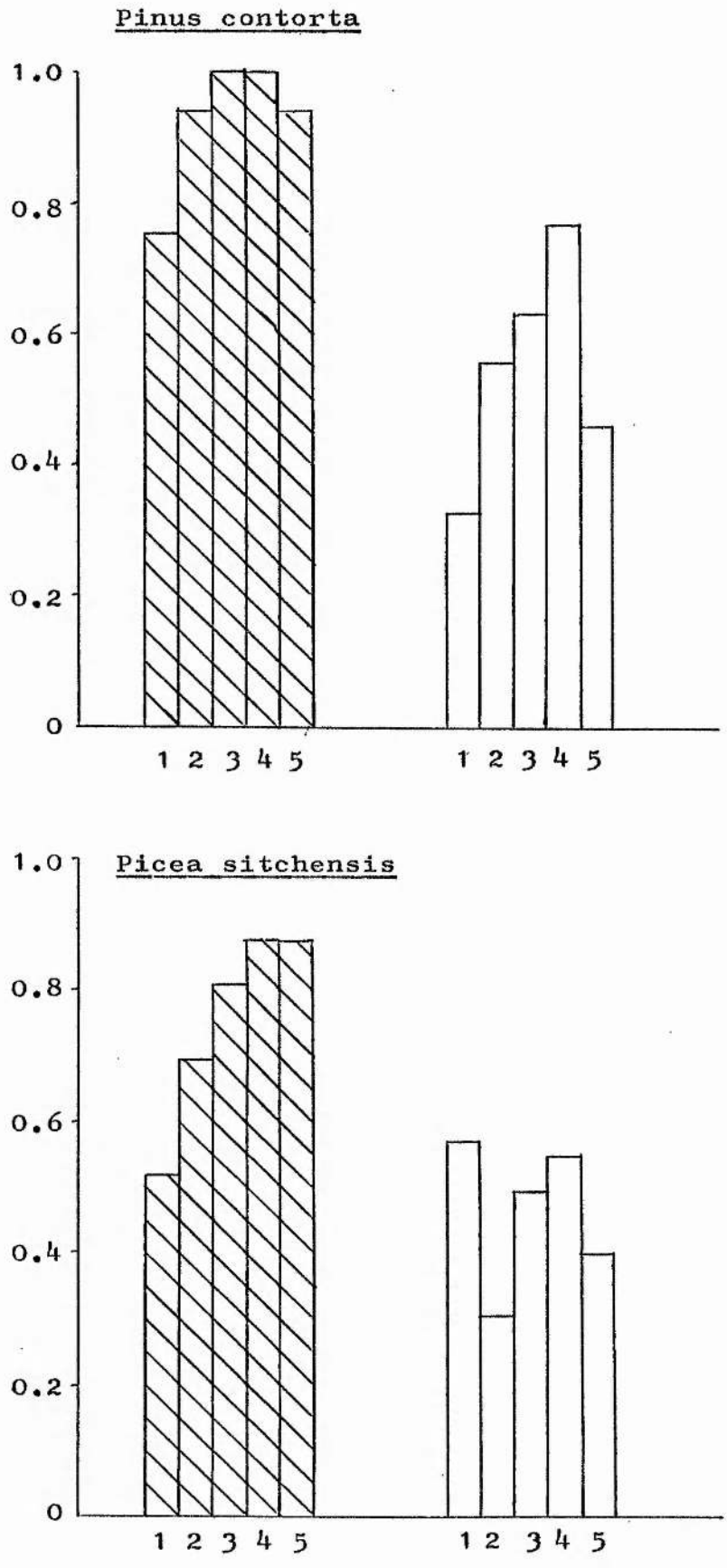


Fig. IV/18

Fig. IV/19

Respiration in terms of RQ (Respiratory Quotient), of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns). Culture vessels also contained added ethanol at various concentrations as indicated.

1 - no added ethanol

2 - 10^{-4}M ethanol

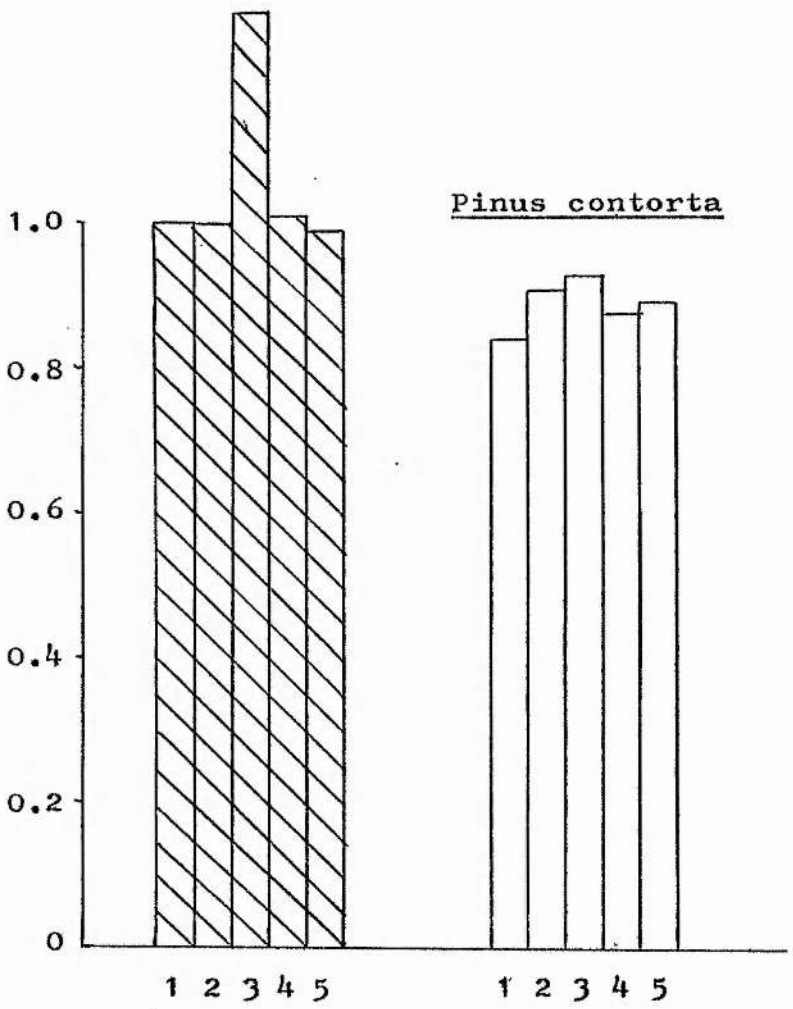
3 - 10^{-3}M ethanol

4 - 10^{-2}M ethanol

5 - 10^{-1}M ethanol

Each column represents the mean value of two separate determinations from roots of two seedlings.

RQ of roots from tree seedlings in water culture with added ethanol



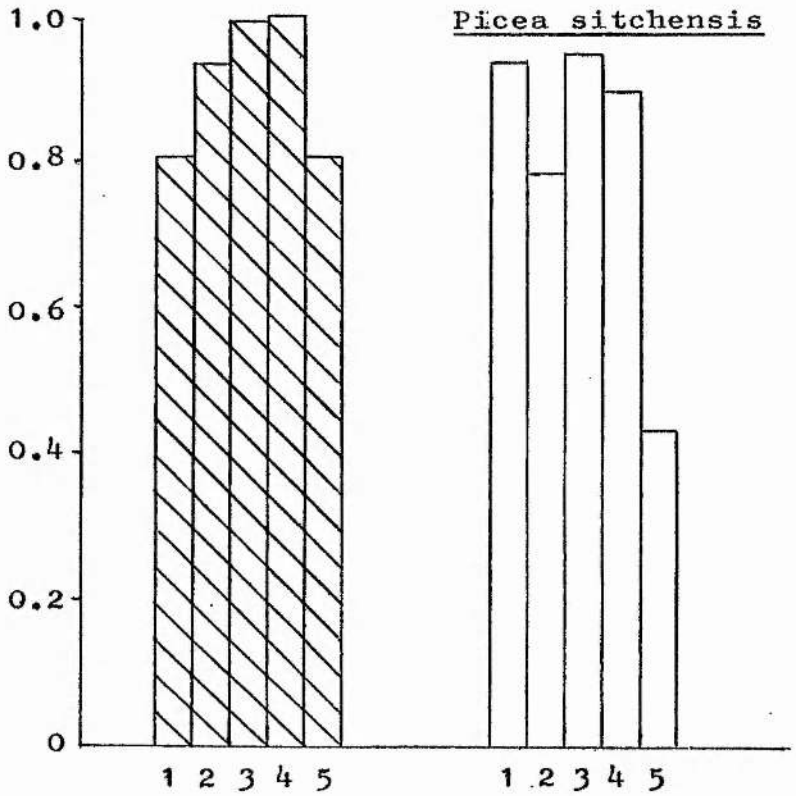


Fig. IV/19

Fig. IV/20

Anaerobic respiration in terms of $\frac{CO_2}{N_2}$, (in µl/hr/mg dry wt), of roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre culture vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns). Culture vessels also contained added ethanol at various concentrations as indicated.

1 - no added ethanol

2 - 10^{-4}M ethanol

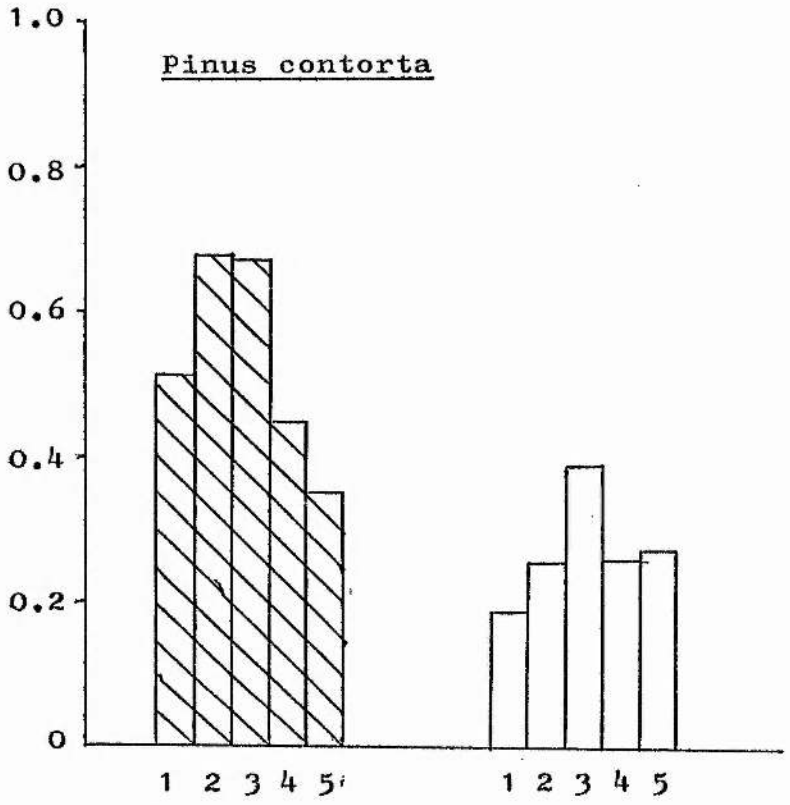
3 - 10^{-3}M ethanol

4 - 10^{-2}M ethanol

5 - 10^{-1}M ethanol

Each column represents the mean value of two separate determinations from roots of two seedlings.

$QCO_2(N_2)$ of roots from tree seedlings in water culture with added ethanol



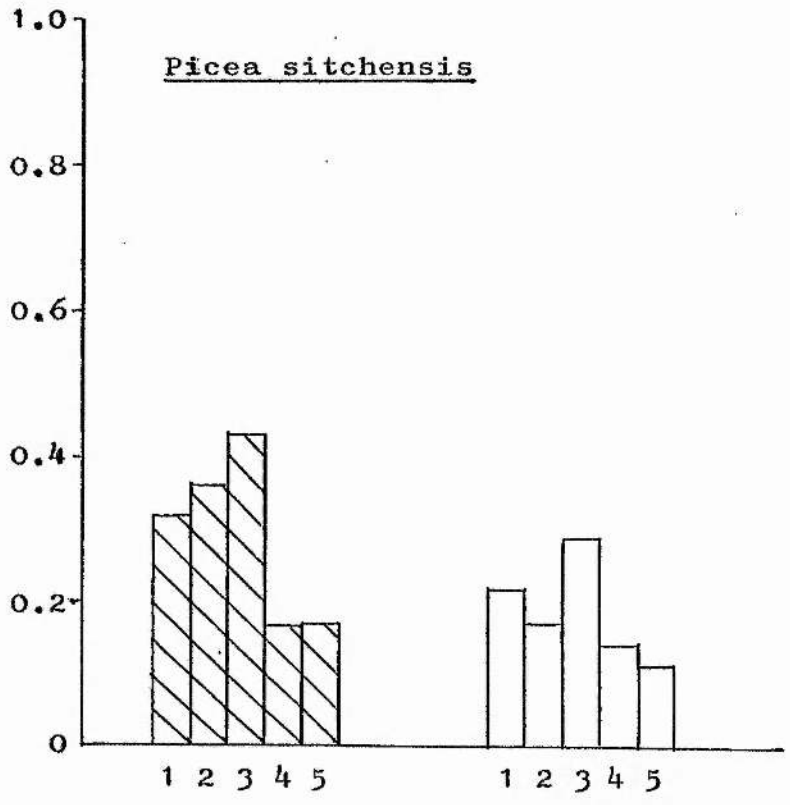


Fig. IV/20

Fig. IV/21

"Pasteur Effect" (see text) as shown by roots from seedlings of Pinus contorta and Picea sitchensis grown in water culture for five weeks in 1 litre culture vessels (Experimental Series F). The water culture vessels were flushed with air (aerobic - hatched columns) or nitrogen (anaerobic - unhatched columns). Culture vessels also contained added ethanol at various concentrations as indicated.

1 - no added ethanol

2 - 10^{-4}M ethanol

3 - 10^{-3}M ethanol

4 - 10^{-2}M ethanol

5 - 10^{-1}M ethanol

Each column represents the mean value of two separate determinations from roots of two seedlings.

"Pasteur Effect" as shown by roots from tree seedlings in water culture with added ethanol

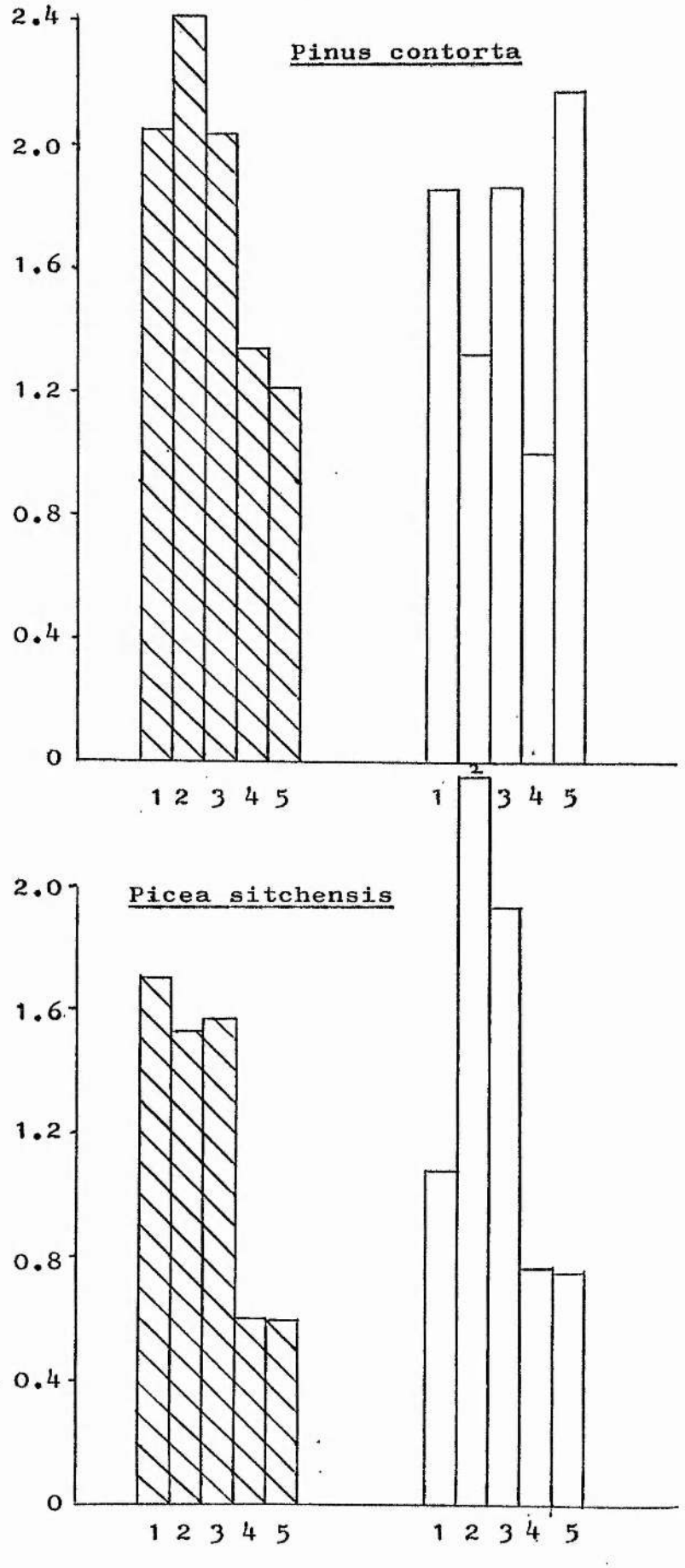


Fig. IV/21

5. B. Discussion

In both species, QO_2 was higher in those roots pretreated aerobically rather than anaerobically. This was also found by Marschner et al (1966), working with corn root tips, where QO_2 was 0.977 with air pretreatment, compared to 0.497 in nitrogen pretreated root tips (in this case, both values were found to be higher if $CaCl_2$ was present in the pretreatment solution). A similar result was also found by Chirkova (1975) for both willow and poplar roots. She found too that addition of ethanol (10^{-2}M) caused an increase in QO_2 in willow from both aerobic and anerobic pretreatments, and attributed this to the ability of willow to reoxidise the ethanol formed. However, she found that the addition of ethanol caused a decrease in QO_2 in poplar, especially if the pretreatment was anaerobic. From this she concludes that poplar therefore cannot reoxidise ethanol. The findings given in this section indicate a similar situation with respect to QO_2 in Pinus contorta, as that found by Chirkova in willow (another flood tolerant tree). The findings for Picea sitchensis do not completely comply with Chirkova's for poplar (another flood intolerant tree). Picea sitchensis plants pretreated anaerobically did suffer an overall decrease in QO_2 with added ethanol, as Chirkova found with both pretreatments in poplar, but in Picea sitchensis, QO_2 increased with ethanol concentration in those plants pretreated aerobically. Chirkova did find that the decrease was less marked in poplars pretreated aerobically.

In this section, QCO_2 was found in general to mirror QO_2; that is QCO_2 was in most cases, higher in aerobically pretreated plants, than in those anaerobically pretreated. This was found to be true for Lathyrus odoratus, Fagopyrum esculentus and Zea mays seeds, by Leach, (1936). He found that the initial low level of CO_2 output observed when seeds were transferred from an anaerobic (N_2) environment to air, was followed by a gradual increase until normal aerobic levels of output were attained. Addition of ethanol caused a general increase in QCO_2 except in anaerobically pretreated Picea sitchensis. Lowe and James (1960), also found that the addition of ethanol to carrot discs in Warburg

flasks (either during or after equilibration) resulted in an increase in CO_2 given off, as well as in an increased uptake of O_2. Unlike Chirkova, however, they conclude that this increased respiration is not due to oxidation of ethanol, since, when the alcohol is labelled, no appreciable labelling occurs in the CO_2 given off, or in the Krebs cycle acids. Later workers (Cossins & Beevers1963; Cossins and Turner 1963) have however, managed to elucidate the pathway of alcohol breakdown in plant tissues. Labelling experiments carried out by Cossins & Beevers (1963) indicate the breakdown of alcohol to acetyl-co-enzyme A, and then by the established pathway.

In both species it was found that roots returned to air after an anaerobic pretreatment, exhibit a lower RQ. Leach (1936) found that this occurred in Lathyrus odoratus, Fagopyrum esculentus, and Zea mays seeds subjected to a nitrogen environment and then returned to air. However, in this case he found that the drop only lasted a few hours, and then rose to the normal aerobic level. The initial drop in RQ was due to an increase in O_2 uptake, which he associated with the oxidation of a substance accumulated during the period of anaerobiosis. In the current investigation however, there is a low level of oxygen consumption on the return to air. The reduced RQ only comes about because this low level of oxygen consumption is still higher (as a percentage of oxygen consumption in aerobic conditions) than the observed low level of carbon-dioxide given off.

$QCO_2(N_2)$ values were lower in roots which had been pretreated anaerobically, and all values were less than those observed for CO_2 in air, for both species. This is again in accordance with the findings of Leach (1936), who reported that seeds pretreated aerobically and then subjected to a nitrogen regime, suffer a reduction in CO_2 output. Khotianovich (1958) found this was so for pine roots.

These results for $QCO_2(N_2)$, and those for the Pasteur Effect, were not those which had been expected, from the results shown in Chapter III. Since,

in that chapter, it had been shown that Picea sitchensis displays a marked increase in ethanol production in anaerobic conditions, it was expected that this would be reflected in an increased CO_2 output and subsequent Pasteur Effect, in both aerobic and anaerobic pretreatments. As can be seen from the results, however, $QCO_2(N_2)$ and, in most cases, P.E., were in fact higher in Pinus contorta (although the highest P.E. observed was that for Picea sitchensis plants with added 10^{-3}M ethanol). A simple explanation for these observations is not apparent.

The calculation of P.E. is based on the comparison of aerobic and anaerobic production of CO_2. If the aerobic breakdown of glucose to CO_2 and H_2O is replaced by the anaerobic breakdown to CO_2 and ethanol, then the CO_2 output is expected to be reduced to one third of that observed in aerobic conditions, for a given amount of glucose. This ratio gives a P.E. of 1 (see previous text). Acceleration of glycolysis (increased consumption of glucose) in anaerobic conditions, is therefore indicated by a P.E. greater than 1. Observation of the QCO_2 (air) for both species, reveals that a much lower level of CO_2 output occurs in Pinus contorta plants taken from an anaerobic atmosphere. This low level may be comparable to that observed by Leach (1936) in similar circumstances where the level did not rise to aerobic levels of output until after several hours. For Picea sitchensis, the CO_2 output (in air) in plants from anaerobic pretreatment is much more comparable to the levels from plants from aerobic pretreatment. This means that the comparison of QCO_2 and QCO_2N_2 in these two plants and pretreatments may be rather misleading. CO_2 output from anaerobically pretreated Pinus contorta roots in N_2 was in fact lower than that from similarly treated roots of Picea sitchensis roots, but on comparison to QCO_2 air, the P.E. is calculated to be higher in this case in Pinus contorta than in Picea sitchensis roots (both pretreated anaerobically). The very fact that CO_2 production is higher in anaerobically pretreated Picea sitchensis roots, even when they are

returned to air, suggests that a higher level of respiration was in progress than in Pinus contorta. If the levels of QCO_2N_2 are compared for each pre-treatment in each species, it can be seen that the ratio of production of CO_2 by anaerobically pretreated roots, to that of aerobically pretreated roots, of Picea sitchensis, is higher compared to the same ratio for Pinus contorta.

However, the overall production of CO_2 in nitrogen was lower than was expected (Crawford 1966). Although the ratio was higher in Picea sitchensis, in no case was the production by anaerobically pretreated plants higher than that in aerobically pretreated plants. It is possible that an unusually high "shock" level of respiration occurs when aerobically pretreated plants are first placed in anaerobic conditions - a level which may be soon reduced. This would make the level in this pretreatment higher than expected, and thus the anaerobic pretreatment levels lower in comparison. However, these levels from the anaerobic pretreatment are still much lower, especially for Picea sitchensis, than had been expected. The results obtained by Crawford (1966), for various species of Senecio indicated that Picea sitchensis, as a flood intolerant plant, would show high production of CO_2 when treated anaerobically, due to an acceleration of glycolysis. However, since the plants discussed in this chapter had been cultured in anaerobic conditions for 5 weeks, a likely explanation for the low levels of CO_2 output, is that the levels of carbohydrates, from which the CO_2 is produced, were very low, and thus respiration was limited. This may also explain the low QO_2 and QCO_2 observed on the initial return to air.

Ethanol can apparently be used as a substrate by both species in aerobic conditions - indicated by the increase in QO_2 and QCO_2 when ethanol is added (Chirkova 1975). It appears that ethanol can also be used by Pinus contorta roots which have been under conditions of anaerobiosis, or are still under anaerobic conditions ($QCO_2(N_2)$), and this is possibly achieved, at least in

part, by the use of nitrates as terminal hydrogen acceptors (Chirkova 1975; Garcia-novo and Crawford 1973; Melsted, Kurtz and Bray 1949; Siegel 1961). However, this is not the case with Picea sitchensis, which does not seem able to utilise added ethanol if the pretreatment is anaerobic. Garcia-novo and Crawford (1973) demonstrated that Picea sitchensis has a much lower capability for using nitrates as alternative electron acceptors than Pinus contorta. Chirkova (1975) observed ADH activity in willow roots during aeration and after 3 days of anaerobiosis. She found that the rate of alcohol oxidation increased in anaerobic conditions, and that the maximal activity occurred at an ethanol concentration of 10^{-3}M, rather than 10^{-2} as found in aerated condition. She concludes that these results probably indicate that there was an increasing affinity of ADH for ethanol following anaerobic conditions in the flood tolerant willow. Maximum activity occurred after 3 days anaerobiosis at an ethanol concentration of 10^{-3}M (affinity lower at higher ethanol concentrations). This was not the case for poplar, where maximum ADH activity, with ethanol as substrate, occurred at 10^{-1}M ethanol (the highest concentration used), but in this case, affinity for low concentrations (10^{-4}M) acetaldehyde was far greater than the affinity for even high concentrations of ethanol. This may have some relevance in the present study, since, for Pinus contorta maximum QO_2 and QCO_2 occurred at a lower ethanol concentration for plants pretreated anaerobically, than for those pretreated aerobically (figs. IV/17 & 18). This may indicate the greater affinity of ADH for ethanol at this lower (usually 10^{-2}M rather than 10^{-1}M) concentration following anaerobic (= "flooding") conditions, thus aiding the oxidation of ethanol at this lower concentration. In poplar, Chirkova found that ADH activity increased with ethanol concentration up to the maximum used, 10^{-1}M, although activity was higher with low concentrations of acetaldehyde as the substrate.

The activity with ethanol as substrate was lower in anaerobic conditions than in aerobic conditions. This may again be reflected in the results of this present investigation for Picea sitchensis. The apparent oxidation of added ethanol occurred only in aerobic conditions, and increased with increasing ethanol concentration up to the maximum used of 10^{-1}M. The activity of the enzyme Chirkova found in poplar was at its minimum with ethanol as substrate and in anaerobic conditions; and at its maximum still in anaerobic conditions, but with acetaldehyde as substrate. In these latter circumstances, it is unlikely that any oxidation of ethanol occurs. In this case, instead, there is a high accumulation of ethanol (see Chapter III), which cannot be utilised in place of the lacking carbohydrate as may occur in Pinus contorta. The situation may be as Chirkova et al (1974) proposes for Phaseolus vulgaris - the high level of ethanol leads to the inhibition of ADH, but high levels of pyruvate (recovered from malic acid by the malic enzyme) prevent the reversal of the reaction. ADH is blocked on both sides. "All this leads to a condition of total disorganisation of metabolism, which .... eventually brings about the death of the plants". This would also render them unable to reoxidise (shown by an increase in QO_2) ethanol even on a return to air. In Glyceria aquatica the flood tolerant species studied by Chirkova et al (1974) in the same paper as Phaseolus vulgaris, the levels of pyruvate, she proposed, remained low, since there was continued formation of malic acid from pyruvate (via PEP and OAA), but no recovery, due to the low level of activity of malic enzyme during anoxia in these plants.

6. GENERAL CONCLUSIONS

The results presented in this chapter do not all lend themselves to straightforward interpretation. This may be partly related to the experimental conditions such as forced aeration (and also forced nitrogen in section 5). The presence of water itself around the roots may be important, even if it is aerated (Kramer 1965; Ohmura and Howell 1960), and Nogodawithana et al (1976) have demonstrated the difficulties encountered when trying to assess the effects of externally applied ethanol. They found that ethanol produced by cells was far more toxic than the same, or smaller quantity of ethanol added to the medium of Saccharomyces cerevisiae during "rapid fermentation". 9.4% (wt/vol) ethanol produced by the cells was much more lethal than even 13.8% (wt/vol) added to the cells.

However, if the results are taken as they stand, what is clear in Pinus contorta is that increasing concentrations of ethanol appear to augment any effects of anaerobic conditions on the plant. This suggests that the major effects that anaerobic conditions have upon these plants may well be effects mediated by the presence of toxic levels of ethanol.

The situation in Picea sitchensis, albeit different and rather unexpected, is equally as interesting. In the experiments discussed in this chapter and in chapter I, dealing with potassium leakage and conductivity of the external medium, Picea sitchensis is consistantly shown to be more affected by aerated conditions than unaerated conditions, even, to a large extent, when external ethanol is applied. These results are consistant but do not agree with other observations and are difficult to interpret. Although they indicate that the Picea sitchensis roots are less affected by anaerobic conditions than Pinus contorta, other results in this thesis (chapter III) and from other workers (Boggie 1974; Coutts & Armstrong 1976), refute this. In this chapter, the

descriptive data from sections 3 and 5 both indicate that Pinus contorta is better able to withstand both anaerobic conditions and external ethanol than Picea sitchensis. There is clearly a need for further investigations into the mechanism of leakage in Picea sitchensis and Pinus contorta. The results may be due to some alternative cause not considered here.

The results of section 5 (respiration), appear to indicate that ethanol added to the medium around the roots of Pinus contorta seedlings, can be oxidised in both aerobic and anaerobic conditions, since QO_2 and QCO_2 increase when ethanol is added (nitrates may be utilised as alternative electron acceptors in anaerobic conditions). Picea sitchensis, it seems can only oxidise ethanol if the medium around the roots is aerated, since QO_2 and QCO_2 are only increased when ethanol is added in aerobic conditions, not in anaerobic. This may also be connected with the apparently greater effect of added ethanol on chlorophyll levels of Picea sitchensis than on chlorophyll levels of Pinus contorta. Ethanol may be transported from the roots to the leaves of Picea sitchensis, where it affects the photosynthetic apparatus, probably by the same mechanism that it affects the roots. It is probable that ethanol reaching the leaves of Pinus contorta would have the same effect, however, if ethanol is being oxidised in the roots of Pinus contorta, little or no ethanol may reach the needles. If ethanol can be utilised in Pinus contorta, apart from removing a potentially lethal product, it may also help to alleviate the "starvation" which may occur during anaerobic treatment. This may occur because of the reduced transport of carbohydrates from the leaves (Vartapetian et al 1977), and also their reduced synthesis in the leaves. This situation is aggravated in plants where glycolysis is greatly accelerated in anaerobic conditions.

Overall, the results of this section seem to indicate that Pinus contorta, a flood tolerant tree, can optimise its conditions by utilising ethanol formed, thereby gaining a source of carbon, whilst also ridding itself of a potentially harmful substance. This provides time for the plants to produce new roots nearer

the soil surface which may be better able to cope with the conditions. It is likely that this ability to utilise ethanol is accompanied by a regulation of the production of this toxic substance (Chapter III).

Picea sitchensis, a flood intolerant tree, appears not to be able to utilise ethanol in anaerobic conditions, and even produces it at an accelerated rate (Chapter III). Thus Picea sitchensis roots accumulate a toxic end product which, if transported out of the roots up the stem, may then affect the photosynthetic apparatus, reducing the source of substrate for which ethanol cannot be substituted, as it can in Pinus contorta. The situation in Picea sitchensis is such then that new, possibly adapted roots are unable to be produced before the demise of the old roots.

DISCUSSION

Anoxic conditions are widespread in nature, partly because there is such great competition for the oxygen available. There is a zone of natural anaerobiosis to be found in the apical 3-5 mm of the root tip, even when the roots are growing in an atmosphere of 21% 02 (Crawford 1976). This is due both to the fact that access of oxygen to the root meristem is hampered by its anatomical structure, and also to the fact that it is a site of intense metabolic activity and utilisation of oxygen. Obviously any reduction in supply of oxygen to the root will extend this zone of anaerobiosis further up the root. Evidence for the increase in anoxic conditions towards the root is also provided by Grineva (1963) who found that the level of ethanol in the root decreases away from the tip, even in anaerobic conditions. Crawford (1976) has found that the Pasteur point ($1\%O_2$) below which anaerobic respiration is of greatest importance, is readily reached in root meristems, even in aerated conditions.

These factors are important because, once the root respiration becomes primarily anaerobic, a number of problems are encountered. Firstly there is the reduction in energy supply to the root. Anaerobic respiration provides only 2 moles of ATP from one mole of glucose, as opposed to the 36 moles of ATP from one mole glucose as obtained from aerobic respiration. This reduction in energy supply may lead to the inability to maintain membrane integrity and the eventual disruption thereby of the complete cellular organisation. However, Kramer (1951b) points out that lack of energy alone cannot explain the rapid flood-damage incurred by some flood intolerant plants. Lack of energy may, however, form part of a self accelerating pattern of events which can occur in anaerobic conditions, and lead to damage. When there is a lack of oxygen, the "oxygen debt" must be stored in a product of anaerobic respiration. The most common end-product, and storer of "oxygen debt" found in the plant world is

ethanol, a substance which is toxic to the plant if stored in any quantity. There comes, therefore, a position where there is decreasing ATP supply, and increasing amount of ethanol. Any reduction in ATP concentration and thus energy charge, is likely to initiate the Pasteur effect (Atkinson 1968; Krebs 1972) in flood intolerant tissues, causing further increases in ethanol. Increased concentrations of ethanol, in its own turn, is thought to result in the disruption of lipid membranes, including those of the mitochondria, and this results in the inhibition of aerobic respiration, decreasing ATP supplies still further.

The action of ethanol on membranes is probably via its lipophillic properties (Crawford 1977). The ethanol is thought to "fluidise" the membranes (Kiyosawa 1975). This membrane damage is thought to result in leakage of electrolytes, amino acids, organic acids and sugars (Grineva 1975) from the cells, and leakage from roots has been noted as a symptom of anoxic root conditions (Christiansen et al 1970; Hiatt and Lowe 1967). In Chapter I, the effect of anaerobic and aerobic root conditions on root leakage from seedlings of Pinus contorta and Picea sitchensis and in Chapter IV, the effects of external ethanol on root leakage from these species was observed. Pinus contorta displayed the results that would be expected if the above hypothesis were true, that is, leakage from the roots was higher in anaerobic conditions, and was increased still further by increasing concentrations of external ethanol. The situation as observed for Picea sitchensis was very interesting. Since Picea sitchensis is less tolerant of flooded conditions than Pinus contorta, the results were expected to be, if anything, more marked. However, it was consistantly shown that aerated water culture had a more detrimental effect on the roots of Picea sitchensis than did unaerated water culture, even when external ethanol was applied. The only explanation found so far for these results is that Picea sitchensis are more easily damaged by the mechanical effect of

aeration. This is not entirely satisfactory, and further investigation is needed to elucidate the situation.

If leakage from plant roots in flooded soil does occur, it may have a secondary effect on the roots. It is known, for instance, that zoospores of Phytopthora are attracted to the zone of exuded ethanol around the roots of Lupins (Allen 1974; Allen and Newhook 1973). This may result in the infection and eventual rotting of the plants in question. The soil microflora are important in another respect. Their presence may markedly contribute to the levels of ethanol found in plants growing in the soil in two ways. Firstly, the presence of a large population of soil microbes creates considerable competition for the oxygen available (Allen 1974), thus accelerating the onset of limiting conditions. Secondly, Huck and Erickson (unpublished) have found evidence to suggest that much of the ethanol found to be present in the xylem sap of tomato, cotton and soybean plants, was in fact microbial in origin. Kenefick (1962) has shown that sugar beet leaves could transpire ethanol supplied externally to the roots. Glasshouse experiments discussed in this thesis in which ethanol levels were determined, all entailed the surface sterilisation of the roots. Thus the contribution of microbes to the ethanol found was greatly reduced if not completely discounted. This was not of course true for the ethanol measurements made from samples taken in the field. However, from the point of view of using ethanol as a measure of soil aeration and/or plant damage, it is largely irrelevant where the ethanol originates from, since its presence indicates inadequate oxygen supply, and if its concentration is high enough, it will cause damage no matter what its origin. It must be remembered that the presence of ethanol indicates an inadequate oxygen supply, but not necessarily low oxygen concentration. The field experiments conducted at Tentsmuir displayed the fact that high ethanol concentrations occurred in the base of the trunk, not only during periods of waterlogging when there was low supply of oxygen, but also during periods of high metabolic activity in the Spring, when

the oxygen available could not meet the demand.

The very presence of ethanol indicates that anaerobic respiration is in progress and that available oxygen cannot completely be meeting the demands of aerobic respiration. It is interesting therefore, to note the widespread occurrance of ethanol in plant tissues, in relation to the contention that, in flood tolerant species, any lack of oxygen in the root environment, may be overcome by transport of oxygen from the overground parts of the plant. Although this mechanism is doubtless very important in some species, and may certainly alleviate the conditions, the presence of ethanol is witness to the fact that internal aeration cannot fulfill the entire oxygen demands of the plants observed in the work discussed in this thesis. However, the evidence discussed here relating to the metabolic differences between the flood intolerant Picea sitchensis, and the flood tolerant Pinus contorta, along with the anatomical evidence found by Coutts and Philipson (1978) and others, combine to give a more complete and interesting view of what processes may really be occurring in these two species when flooding occurs.

During a gradual rising of the water table there is the formation of airways through the trunk to the roots of Pinus contorta. The oxygen supplied by these airways enables the survival of shallower roots, perhaps even limited growth into the water table, and the formation of adventitious roots near the surface. Survival of sudden flooding, and of the deeper roots, cannot depend on the formation of these air spaces, but their prior existence and the resultant higher residual of oxygen remaining in the root, may be required to allow time for the induction of metabolic change to come into force. This metabolic change entails some form of control of the rate of glycolysis, and control of the production of ethanol. The low concentration of ethanol recorded in flood tolerant species is due partly to this control of ethanol production, but perhaps also

to oxidation of the ethanol formed, either by nitrates, or by oxygen from the shoot, which may thus perform a dual function. Oxidation of ethanol serves not only to reduce the concentration of a toxic substance, but also provides an additional carbon source at a time when normal sources may be low.

In the flood intolerant Picea sitchensis there is no evidence for internal aeration or control of glycolytic rate. In this case there is actually an increased production of ethanol. There is no evidence for internal oxygen supply, so that it is unlikely that the situation can even be alleviated by the oxidation of ethanol. In the case of the flood intolerant plant the roots are killed very quickly, allowing no time for the production of shallow adventitious roots near the surface.

Although this presents a much fuller picture of the processes in action, it is still likely to be far from complete. There is evidence, for instance, that some flood tolerant plants may be more tolerant of high ethanol concentrations. Chirkova (1975) found that willow roots were still viable in 10^{-1}M ethanol after 6 days, whereas poplar roots were killed by the second day. There is also evidence from a number of flood tolerant species, that tolerance of flooding is linked with end product diversification (Crawford 1976). In these cases, non-toxic end products such as malate and lactate (Crawford & Tyler 1969; Crawford 1977), and amino acids (Dubinina 1961), are formed, which may be stored at quite high concentrations without harm. Davis (1973) has suggested that accumulation of organic acids is limited by tissue acidification, since the differing pH optima of carboxylating and decarboxylating enzymes will act as a pH stat. However, organic acid accumulation will not be reduced by pH reduction if the acids are stored in the vacuole.

Accumulation of various organic acids by plants under conditions of complete or partial anoxia, has been observed by a number of workers, and reviewed by Crawford (1972). Work in this laboratory (Baines unpublished) on various wetland species has shown the accumulation of malate, lactate and to a lesser extent,

glycerol, as well as ethanol. The differential accumulation of malate by flood tolerant and flood intolerant species was shown by Crawford and Tyler (1969) and was later extended by Linhart and Baker (1973) who showed this was true for two varieties of the same species, Veronica peregina. Plants taken from the centre of a pool showed significant increases in malate when flooded, whereas those from the edges of the pool did not. McManmon and Crawford (1971) initially suggested that the accumulation of malate is made possible by the deletion of the malic enzyme on the roots of flood tolerant species. It has since been shown, however (Chirkova et al 1974; Davies et al 1974) that malic enzyme is in fact present in various flood tolerant species, but that its activity is reduced when flooded and increased by aeration. By contrast, in species intolerant of flooding, exposure to anaerobiosis caused an increase in malic enzyme. Sarawek and Davies (1976) found that in flood tolerant species, the activity of malic enzyme is inhibited by oxalic acid.

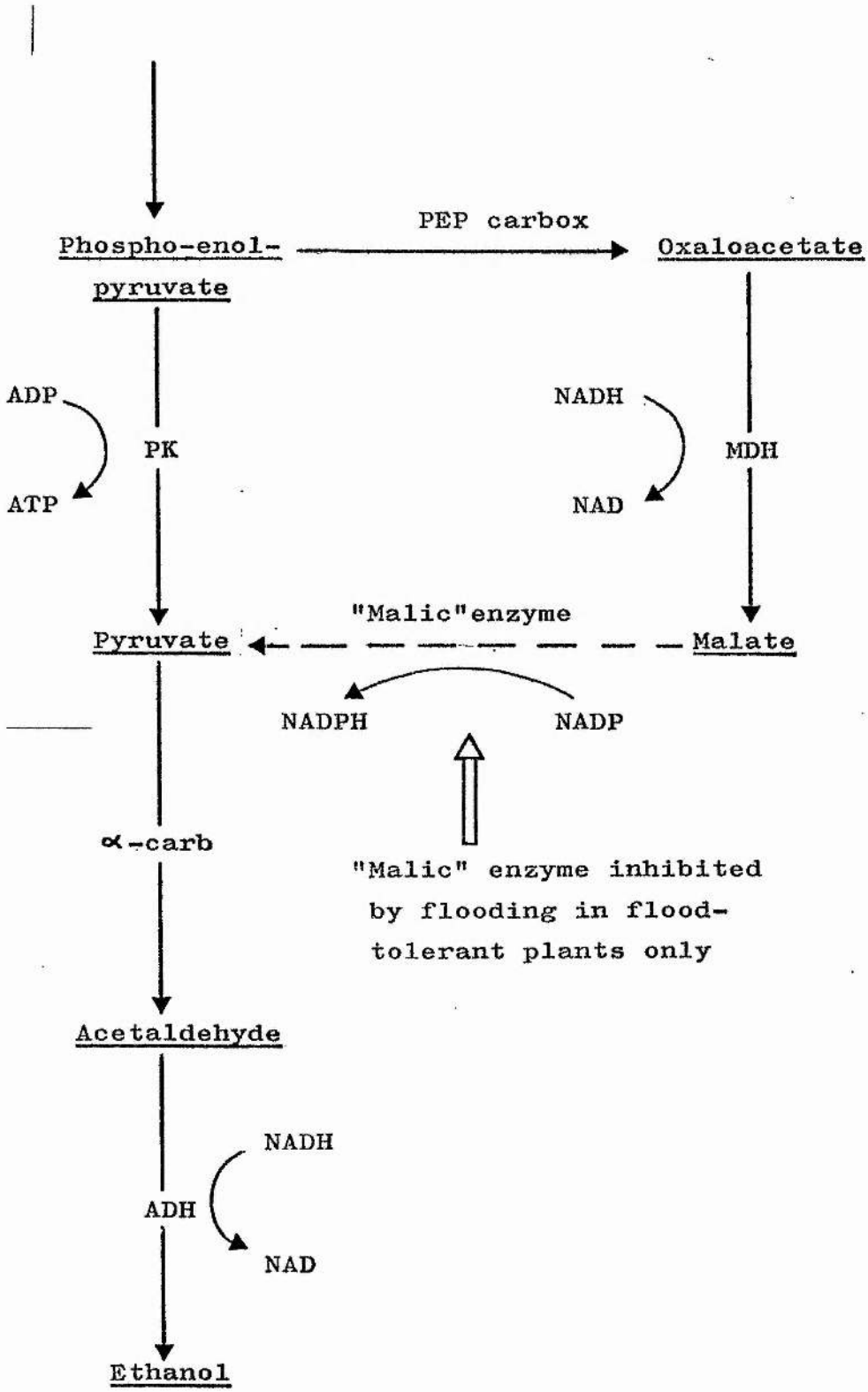
Crawford's "metabolic theory of flooding tolerance" (McManmon & Crawford (1971) is outlined in fig. D/1. He suggests that in "intolerant" roots, on flooding, normal respiration is blocked, and glycolysis proceeds to the production of acetaldehyde and ethanol. Acetaldehyde induces alcohol dehydrogenase activity which together with a reduction in apparent Km value, accelerates glycolysis. Malate present is decarboxylated by malic enzyme to pyruvate and thence to acetaldehyde, contributing further to ethanol production. Oxaloacetate and hence malate may be formed by the carboxylation of phosphoenolpyruvate, but the malate will not accumulate. Ethanol and acetaldehyde do accumulate and contribute to the poisoning of metabolism. In "tolerant" roots on flooding, normal respiration is at least partially blocked, and glycolysis may proceed to the production of acetaldehyde and ethanol, but the former fails to induce ADH activity, the apparent Km value remains unchanged, and no acceleration of glycolysis ensues. Malate present is not decarboxylated because

Fig. D/1

"A Metabolic Theory of Flooding Tolerance" from McManmon and Crawford (1971)

PEP carbox	-	Phospho-enol-pyruvate carboxylase
MDH	-	Malate dehydrogenase
PK	-	Pyruvate kinase
-carb	-	- carboxylase
ADH	-	Alcohol dehydrogenase

"A Metabolic Theory of Flooding Tolerance"



(McManmon and Crawford 1971)

Fig.D/1

malic enzyme is inhibited. Oxaloacetate and hence malate are produced by the carboxylation of phosphoenolpyruvate, and malate accumulates. This is non-toxic and may remain without harm to the plant until aerobic conditions are restored.

Since it appears that there are several possible products of anaerobic respiration, the question naturally arises as to whether there is an alternative product, rather than ethanol, which could be used as a monitor of oxygen stress. An alternative in the form of GAB has been discussed by Huck and Erickson (unpublished). However, this was found to be even more liable to environmental change than ethanol. Although there are many other possible products of anaerobic respiration, the primary and the most ubiquitous, is ethanol, and it is the only one which is produced exclusively by the process of anaerobic respiration. The characteristics of ethanol, and the restricted conditions under which it is accumulated make it an ideal candidate for use as a test of oxygen stress, and also of the degree of flood tolerance of the plant concerned. The results discussed in this thesis show great promise for the development of the use of ethanol as such a monitor. There are very many problems to be dealt with before it can be brought into use on a day to day basis, but the results from this limited trial are very encouraging for the future.

SUMMARY

The initial aim of this thesis was to investigate the various indications and symptoms of flooding damage found in coniferous trees, and to assess their possible use as monitors of the effects of flooding.

1) Root leakage under anoxia was investigated, using seedlings of Pinus contorta and Picea sitchensis in water culture. Anaerobic conditions were found to increase leakage, (in terms of increasing potassium content and conductivity of the surrounding external culture solution), from roots of Pinus contorta. However, aerobic root conditions were found to cause more leakage from Picea sitchensis roots, than anaerobic conditions. This may possibly be due to the mechanical effect of aeration. pH of the medium was found to increase in aerobic conditions and decrease in anaerobic conditions, for both species.

The possible causes of leakage are discussed.

2) Chlorophyll levels were determined for needles from Pinus contorta and Picea sitchensis seedlings in aerobic or anaerobic water culture. Aerobic conditions again appeared to have the more detrimental effect on Picea sitchensis.

3) Ethanol levels were determined of wood cores taken from trees of Pinus sylvestris growing in sites of varying wetness, at Tentsmuir, Fife. Samples from trees growing in the wetter areas were found to contain more ethanol than samples from trees growing in the drier areas.

On the basis of the above information, it was decided that of the symptoms of flooding damage investigated, ethanol content appeared to be the most likely candidate worth investigating for use as a monitor of anaerobic conditions and flooding damage. It can also be developed to involve a simple field sample collection procedure. The high levels of ethanol found in flooded trees may, in addition, provide a clue as to the nature of flooding damage.

4) Seasonal fluctuations in ethanol content of wood cores from trees of Picea abies (from one site) and Pinus sylvestris (from two sites) at Tentsmuir, Fife. Samples were taken at monthly intervals throughout the year, and their ethanol content determined. Ethanol could be detected throughout the year. Good correlation was found between ethanol levels in individuals of the same species on the same site, and a similar pattern of ethanol levels throughout the year was displayed by different species on different sites. Ethanol levels were highest during the wet autumn-winter period, but also during the Spring, when there was high metabolic activity and increased oxygen demand. The possible use of ethanol as a monitor of soil and plant conditions is discussed, and its use as an indicator of the ability of a tree to withstand flooding.

5) Anoxia in water culture, ethanol production and flooding tolerance

Ethanol levels were determined for roots of seedlings of Pinus contorta and Picea sitchensis, maintained in aerobic or anaerobic water culture, and in flooded or unflooded pots. Results -

a) An immediate increase in ethanol concentration in roots of both species on cessation of aeration.

b) Much greater percentage increase (12 fold after 24 hours) in ethanol in the flood intolerant Picea sitchensis.

It was suggested that high levels of ethanol have a toxic effect, possibly related to its lipophillic properties.

6) The toxic nature of ethanol - investigated by maintaining seedlings of Pinus contorta and Picea sitchensis in aerobic or anaerobic water culture, with added ethanol from 10^{-4}M to 10^{-1}M ethanol.

a) Leakage from roots with external ethanol.

Pinus contorta - increasing leakage with increasing ethanol concentration

Picea sitchensis - effect of external ethanol was not very clear. Maximum leakage again appeared to be from roots in aerobic conditions (no added ethanol).

Overall leakage was higher from Pinus contorta. External ethanol (in anaerobic conditions) caused a further decrease in pH of the culture solution for both species, but the effect of ethanol was more marked in Pinus contorta.

b) Root tip viability was assessed using 2-3-5 triphenyl tetrazolium chloride stain, after immersion of the root systems of seedlings of Pinus contorta and Picea sitchensis in ethanol (2×10^{-2}M to 2M) for 7 days. 2M and 2×10^{-1}M ethanol killed roots of both species after 7 days. Most roots of both species were also moribund in 2×10^{-2}M ethanol, but some new roots were present in Pinus contorta in this concentration.

c) Chlorophyll levels were determined of needles from seedlings of Pinus contorta and Picea sitchensis with roots immersed in 10^{-4}M to 10^{-1}M ethanol for 14 days. The effects of external ethanol were more marked in Picea sitchensis than in Pinus contorta (in contrast with the results for leakage).

d) Respiration rates of roots in ethanol. QO_2, QCO_2, $QCO_2(N_2)$, RQ and Pasteur Effect (P.E.) were found for root segments taken from Pinus contorta and Picea sitchensis seedlings maintained in culture solutions containing 0, 10^{-4}M, 10^{-3}M, 10^{-2}M, or 10^{-1}M ethanol, bubbled with air or nitrogen, for a five week period.

QO_2 was always higher in roots pretreated aerobically. Addition of ethanol caused an increase in QO_2 in aerobically pretreated plants of both species, and anaerobically pretreated plants of Pinus contorta. It was suggested that this increase was due to the oxidation of ethanol, and that , although Pinus contorta could oxidise ethanol in anaerobic conditions, Picea sitchensis could not. QCO_2 generally mirrored QO_2. RQ was lower for anaerobically pretreated roots.

$QCO_2(N_2)$ values were also lower for anaerobically pretreated roots, and were less than values for QCO_2. $QCO_2(N_2)$ and P.E. values were expected to be higher than they were, especially for Picea sitchensis, based on the observations in (5) above. However, CO_2 output from anaerobically pretreated roots of Pinus contorta in nitrogen ($QCO_2(N_2)$ was lower than that from Picea sitchensis in similar conditions, but on comparison to QCO_2 in air, P.E. is deduced to be higher for Pinus contorta.

The widespread occurrance of anaerobic root conditions and the problems caused by anoxia are discussed.

Evidence from this thesis and from elsewhere combine to give a more complete picture of the processes occurring in flood tolerant species such as Pinus contorta. In this case, tolerance of flooding is thought to be due to a combination of the abilities to transport oxygen from the shoot to the root, to oxidise ethanol formed (providing an additional carbon source, as well as a method of detoxification), and to control the actual rate of glycolysis and ethanol production. The flood intolerant species, Picea sitchensis does not possess any of the above abilities, and actually suffers an increased rate of glycolysis and ethanol production, when subjected to anoxic root conditions.

The use of ethanol as a monitor of root conditions and as an indicator of flooding tolerance is discussed. The results of this thesis show great promise for the use of ethanol in the future, in such a way.

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